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Secretary-Editor

University of Michigan,
Ann Arbor, Michigan.

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PAUL S. WELCH

ANN ARBOR, MICHIGAN

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GLARIDACRIS CATOSTOMI GEN. NOV., SP. NOV.: A CESTODARIAN PARASITE

By A. R. Cooper

INTRODUCTION

In a preliminary paper Ward (1911) stated that he had found in fish from the Illinois River a cestodarian tapeworm which showed certain features common to the well known European genera, *Caryophyllaeus* and *Archigetes*. "It resembles the former in the absence of a caudal appendage and in the location chosen by the adult parasite, viz., the intestine of a fish, whereas, so far as known in Europe, *Archigetes* always possesses a tail and has been found only in the body cavity of tubificid worms. In general appearance and structure the American form resembles the European *Archigetes* very strongly. It has a scolex of fixed form with prominent suckers or phyllidea and also the musculature of *Archigetes*. The general arrangement of the reproductive organs, especially the two rows of testes in the central field, and the genital pores, correspond also closely to conditions in *Archigetes*." Much later the same writer (Ward and Whipple, 1918) merely stated in his key to the Cestodaria that, as regards *Archigetes*, "a form which undoubtedly belongs here has been described to me as found in native earthworms." Neither under *Archigetes* nor under *Caryophyllaeus* does he make any further mention of the above form, and concerning *Amphilina* says only: "Not yet reported from North America but present." Nor have I been able to locate any other reference to members of the Cestodaria, sensu latu, having been found on this continent up to date.

Before proceeding with the detailed account it should be mentioned as a matter of introduction that, apart from being evidently

the first member of the group to be described from America, the species to be dealt with here is of special interest in that it seems to stand intermediate in the family, Caryophyllaeidae Lühe 1910, between *Archigetes* and *Caryophyllaeus*. Excepting for the scolex, however, which is quite similar at least in outward appearance to that of *Archigetes brachyurus* Mrázek, it closely resembles the species of *Caryophyllaeus*, of which three, namely, *C. laticeps* (Pallas), *C. tuba* (Wagener) and *C. fennicus* Schneider, have been found in Europe, and one, *C. syrdarjensis* Skrjabin, in Asia (Turkestan).

MATERIAL

The material for the present study was obtained at the Douglas Lake Biological Station of the University of Michigan during the summer of 1917 while the writer was paying particular attention to the bothriocephalid cestodes of fishes. In all thirty-six specimens of *Catostomus commersonii* (Lacépède), the host species, were examined. These fell into two lots as regards size: ten younger ones ranging in length from 90 to 115mm. and twenty-six adults from 250 to 325mm. The latter were caught in the trammel and fyke nets used in the lake proper, while the former were seined out of Maple River which drains the lake. No parasites belonging to the species described here were met with in the younger hosts, but from two to at least sixty-three were found in the stomachs and intestines of eleven of the adults. The table shown on page 7 gives their number, distribution and kind in nine of the hosts, the exact numbers not having been recorded for the other two fish.

From this it is seen that the degree of infestation of the host is comparatively small. Whereas the number of adults met with was quite limited, larvae were very plentiful when present at all. In situ all of the adults and most of the larvae were found free in the stomach or intestine, but many larvae—forty-one in the case of the third fish in the table—were attached to the bottoms of deep pits in the mucosa of the pyloric region of the stomach. These pits were not mere depressions of the wall of the stomach but actual cavities, as shown in figure 7, bordered by a pronounced annular thickening of the mucous membrane and as much as 2mm. in diameter. Larvae ranging in size from almost the smallest met with to those near the adult stage in development were tightly crowded into these pits and at the same time strongly contracted longitudinally.

Length of host	Stomach		Intestine	
	Number	Kind	Number	Kind
285 mm	9	Adults	.....	.....
250 mm	4	Adults	.....	.....
	14	Larvae	.....	.....
275 mm	63	Larvae	.....	.....
313 mm	.....	.....	2	Larvae
282 mm	9	Larvae	52	Larvae
295 mm	.....	.....	2	Adult
			1	Larva
295 mm	7	Larvae	21	Larvae
280 mm	.....	.....	20+	Larvae
265 mm	.....	.....	12+	Larvae

EXTERNAL FEATURES

On account of its possessing a well developed musculature for its size this species exhibits considerable differences in degree of contraction and elongation on fixation. If no care is taken in applying the fixing reagent nor in slightly manipulating the specimen, it usually contracts to such an extent that it becomes almost useless, at least for the making of toto preparations. However, the adults, which are here considered to be those whose uteri may be seen in toto preparations to contain a few or many eggs, may be said to range in length from about 5 to 25mm. and from 0.4 to 1.0mm. in maximum breadth.

In immature individuals the scolex, when not strongly contracted, has somewhat the form of a truncated rectangular pyramid with the longer diameter in the transverse direction. As shown in figures 1 and 2, the edges of the base and the apex protrude markedly, in the latter case forming a terminal disc comparable to that of many of the bothriocephalid cestodes. The dorsal and ventral faces of the organ are each divided by two ridges converging towards the apex into three sucking grooves or loculi, of which the middle is best developed and most efficacious during life. It is also the last to become smoothed out with strong contraction of the whole scolex. The lateral loculi are, furthermore, not in the same plane with the medial one but inclined towards the corresponding ones of the opposite surface so that the edges of the scolex, especially just behind the

terminal disc, are often not much thicker than the ridges between the loculi. As regards these features the organ consequently resembles that of *Archigetes brachyurus* Mrázek 1908, which is here reproduced (Fig. 5) for the sake of comparison. In adults, on the other hand, the edges of the terminal disc are usually found in preserved material to be contracted to the point of obliteration, so that the whole organ is shaped more like a wedge or chisel with oftentimes rather thick margins (Figs. 3, 4 and 9). As a matter of fact the scolex of this form assumes a greater variety of shapes than that of any other tapeworm I have yet examined, in which respect it is comparable to the leaf-like anterior end of *Caryophyllaeus*. The dimensions of the organ are as follows: Length, 0.30 to 0.45mm.; width (posteriorly), 0.45 to 1.10mm.; depth (posteriorly), 0.50 to 0.75mm.

Behind the scolex the strobila narrows down for a short distance and then much more gradually enlarges again to the region of maximum diameter, which is usually behind the genital openings. Yet in many specimens, especially the more relaxed ones, the whole strobila is all but uniform in width thruout its length. The region between the scolex and formost vitelline follicles, which includes the narrowest portion of the strobila and is consequently called the neck, varies from 1.5 to 2.5mm. in length. Finally the posterior end, as shown in figure 6, is somewhat triangular in outline with a slightly indented tip where the excretory vessels open to the exterior, but bears nothing in the nature of an appendix such as it present in *Archigetes*.

CUTICULA, SUBCUTICULA AND PARENCHYMA

The cuticula, which varies in thickness from 7 to 11μ, is bounded on the inside by a comparatively heavy basement membrane, about one-sixth of the thickness of the whole layer, and on the outside by a smooth membrane about one-half as thick as the basement membrane. The remainder of the tissue has the appearance of a reticulum enclosing numerous distinct granules. This reticulum is in reality a meshwork of fine canaliculi which freely pierce both limiting membranes, thus giving them the appearance in tangential sections of fine sieves. Nowhere is the cuticula modified to form spinelets nor distinct cirri, altho over the scolex it is considerably folded and

irregular, the outer membrane being all but absent, especially within the suckers. For *Caryophyllaeus laticeps* Will (1893) described a cuticula 5 to 6μ in thickness and composed of only two layers, an outer showing radial striping, as if formed by fine bristle-like hairs, and an inner, more deeply staining stratum, comparable to the basement membrane of this form. He saw no distinct pores in the cuticula, and thought that perhaps the striations might represent prolongations of the subcuticula.

The subcuticula is made up of large flask-shaped cells, closely crowded together and provided with comparatively large nuclei. Whereas the individual cells are not distinctly separated from one another, the whole layer, from 90 to 100μ in thickness, is clearly marked off from the underlying parenchyma owing to the very granular nature of its components. The nuclei, which are spherical to oval in shape and provided with distinct spherical nucleoli, vary from 16 to 18μ in greatest diameter. They are located at different levels, so that the whole layer has a pseudostratified appearance. The enlarged central ends of the cells are usually rounded off towards the parenchyma, which feature is clearly indicated by their characteristically large granules. On the whole the subcuticula is not very different from that of *C. laticeps* as described by Will.

The parenchymatous cells form an open reticulum showing only a very few nuclei. They are in strong contrast with the subcuticular cells on account of their clear, non-granular cytoplasm. Posteriorly the whole tissue is much limited in amount by the large reproductive organs which are imbedded in it. No such "fibrous strands" of modified parenchymatous cells, as described by Will for *C. laticeps* and by Skrjabin for *C. syrdarjensis*, were seen in this form. In the base of the scolex and in the neck region, however, the medulla is occupied by a more or less X-shaped mass of cells (Fig. 10) containing large nuclei with numerous large granules which have a great affinity for the counterstain. They are probably glandular in their nature since they send long processes, especially in the diagonal direction, to the cuticula covering the scolex, between the cells of the subcuticular layer. Furthermore, no evidence of the presence of calcareous bodies in the parenchyma was met with in an examination of both fresh and preserved material.

MUSCULATURE

The musculature is comparable to that of the cestodes proper in that it is composed of two sets of fibres, the parenchymatous and the cuticular. The former consists of sagittal (dorsoventral), frontal (transverse) and two sets of longitudinal fibres, of which the latter are much the strongest. Whereas both sagittal and frontal fibres are few in number, they are not equally so, for the sagittal are somewhat larger and more numerous. Both kinds tend to course slightly obliquely where they are greatly interfered with by the reproductive organs. The main or inner longitudinal fibres are, on the other hand, comparatively large and arranged in thick bundles (Figs. 11, 12 and 13). They are situated among the central ends of the subcuticular or just within them, the cortical parenchyma being thus considerably restricted in amount. Posteriorly the fasciculi are very unequal in size and quite numerous. As they are followed forward, however, their numbers diminish while their size increases, until at the base of the scolex there are only eight large bundles arranged as in figure 10. This is brought about by the fusion of the smaller bundles and the passage of the fibres from one fasciculus to another. In longitudinal sections the bundles are irregularly striated owing to there being a considerable amount of myoplasm in the middle of each fibre around the remains of the original myoblastic nucleus. Nevertheless, no distinct nuclei such as described and figured by Will for *C. laticeps* were seen. In the posterior end of the worm many of these longitudinal muscles terminate in the walls of the excretory invagination or run alongside of it to the extremity of the strobila. The outer longitudinal group (Fig. 10) consists of a large number of bundles, smaller but more uniform in size than those of the inner group, situated among the peripheral ends of the subcuticular cells just outside of their nuclei or from 15 to 30μ from the cuticula. Posteriorly only a few of them pass beyond the anterior end of the excretory invagination, but anteriorly they are very pronounced and continue into the scolex. Similar fibres in *C. laticeps* were considered by Will to belong to the cuticular instead of to the parenchymatous series.

The cuticular muscles consists of an outer stratum of circular fibres lying close to the inside of the cuticula and an inner of longitudinal fibres situated close within that. The longitudinal fibres, which in some places intermingle slightly with some of the outermost

members of the outer longitudinal parenchymatous group, are arranged in small bundles, each containing at most only about ten or a dozen fibres. In the posterior end of the worm they proceed farther back than the latter, after being closely associated with them opposite the excretory invagination. The same may be said of the circular cuticular muscles, excepting that they are not distinctly arranged in bundles.

In the scolex the cuticular muscles are much less pronounced over the sucking-grooves than on the lateral faces. As shown in figure 9, the eight large bundles of inner longitudinal muscles, mentioned above, are arranged so that four form two sagittal pairs situated towards the lateral faces, while the other four, somewhat larger ones form two other sagittal pairs, each about half way between the nerve trunk and the median line. These are distributed in a radiating manner to the corresponding portions of the tip of the scolex, the median pairs going to the ridges between the loculi and the neighboring parts of the latter. On the whole their attachment is similar to that of the main longitudinal group in *C. tuba* and *C. laticeps*, as described respectively by Monticelli (1892) and Will. The outer longitudinal muscles are more numerous on the lateral surfaces of the scolex than opposite the suckers, to the cuticula of which they are easily traced. The loculi are also provided with a few scattered radiating fibres, lying in both the longitudinal and the transverse directions, and comparable to those used in the Pseudophyllidea for the enlargement of the bothria. They are, however, of much less functional importance in that connection than the sagittal and transverse fibres, which are somewhat larger and more numerous than in the middle of the worm. In fine, the musculature of the scolex is poorly developed as compared with that of *Bothriocephalus*, s. str., for example, which fact is shown in the great diversity of shapes of the organ in preserved material. In fact it might be considered to represent an intermediate stage between that of the anterior end of *Caryophyllaeus* and that of the typical bothriocephalid scolex. But the comparative inefficiency of the individual sucking-grooves is compensated for by their number and by their manner of attachment to the host's alimentary tract, namely at the bottom of the spacious pits described above.

NERVOUS SYSTEM

The nervous system consists of a pair of ill-defined longitudinal trunks and two equally indistinct and diffuse terminal ganglia situated in the scolex, into which they pass. The main strands can be followed more or less easily in material not especially treated to demonstrate them only in the neck region. There, as shown in figure 10, they are situated symmetrically in the median frontal plane within the trapezium formed by the two pairs of main longitudinal muscle bundles, much closer, however, to the lateral pair than to the more median pair. They supply these muscles with large branches. Whereas in the neck they are fairly uniform in diameter—which varies from 18 to 30μ—behind the most anterior vitelline follicles they become quite irregular in transection, all but disappearing in places. In the middle of the worm and posteriorly they seem to break up into a diffuse plexus lying just within the subcuticular cells, that is, among the numerous bundles of the inner longitudinal muscles. No collateral strands such as the eight described by Will for *C. laticeps* were seen in this form.

In the base of the scolex these chief nerve strands expand considerably in the dorsoventral direction and become united by a few transverse fibrils. Farther towards the tip, however, each of these enlargements divides into two parts sagittally, and each of the latter unites with its fellow of the opposite side by a loose strand of transverse fibrils, so that two anteriorly directed loops are thus formed. On the whole the nervous system is comparatively poorly developed, since not only the chief strands but also their connections in the scolex are composed of very fine, indistinct and loosely arranged fibrils.

EXCRETORY SYSTEM

Thruout most of the length of the worm the excretory system consists of a single layer of comparatively large and much coiled longitudinal vessels situated just outside of the inner longitudinal muscles among the central ends of the subcuticular cells. Whereas the number of these vessels cannot be stated definitely, owing to many transverse connecting channels, there is a tendency, especially in the anterior regions, for eight of them to take the courses indicated in figure 11. Three are located on each surface and one in the median frontal plane at each side. In the anterior part of the neck region

the number increases, and the courses of these vessels become irregular, that is, the plexus becomes more diffuse. There they invade all parts of the subcuticula and the periphery of the cortical parenchyma (Fig. 10). From 1 to 1.5mm. behind the tip of the scolex two branches leave the plexus above and below the nerve cord on each side (Fig. 10) and unite on the medial side of the latter to form one vessel. In these positions the two vessels thus formed pursue spiral courses forward and apparently unite close behind the nerve commisures mentioned above. For *C. laticeps* Fraipont (1880) and Will described an excretory system consisting in brief of four "ascending canals" and ten "descending canals," connected in the mobile anterior end of the worm with each other and posteriorly with the so-called excretory vesicle. Thus it is seen that as regards the main channels of the excretory system at least this species is somewhat less complicated in structure than the European species in question. In the posterior end of the former the plexus just described converges towards the centre of the medulla, as the vessels diminish in size, and unites by several openings with the terminal receptacle. The latter, as pointed out for *C. laticeps* by Steudener (1877), is merely an invagination of the hinder end of the worm, about 0.25mm. in length by about 0.05 in diameter. Its wall is composed of only a lining of cuticula continuous with that covering the posterior end of the worm and also traceable for some distance into the larger branches leading from the plexus into the invagination. In the sections made it was also seen to be quite vacuolated and granular and poorly provided with cuticular muscles, thus indicating that the whole structure is not a true pulsating vesicle.

Nowhere in any of the sections studied was I able to find the typical terminal organs of the excretory system, namely, the flame-cells, which according to Fraipont are present in *C. laticeps* with the same structure as those in trematodes. But in their place there appeared much less specialized cells which are, nevertheless, comparable in some respects to the ciliated funnels of other cestodes. As shown in figure 8, each consists of a large cell provided with a large nucleus with a distinct spherical nucleolus but much vacuolated cytoplasm. The cytoplasm is aggregated close around the nucleus, and from this mass numerous strands pass to the wall of the cell. The latter is directly continuous with one or more canaliculi which lead off from the structure and connect up with the larger vessels

to form the plexus. The whole has the appearance of an enlargement of the terminal vessel, enclosing an amoeboid cell which is suspended in the centre of the vesicle by its pseudopodia. Thus the vacuolated space which surrounds the cytoplasmic mass and is continuous with the cavity of the canaliculi is comparable in part at least to the funnel which accommodates the "flame" in the typical flame-cell. These terminal organs are situated close around the canals in the periphery of the cortex or even farther out among the inner ends of the subcuticular cells. Furthermore, they are much more numerous in the neck region than elsewhere. The only reference I have been able to find to structures at all comparable to these peculiar cells is that by Wright and Macallum (1887) on *Sphyranura osleri*. For this form, a monogenetic trematode, they described as the terminal renal organs peculiar elongated, club-shaped cells which are situated in close proximity to the vitelline follicles and the principal groups of muscles. The cytoplasm of the cell is divided into a number of coarse, granular trabeculae radiating from the nucleus to the wall, thus leaving a system of communicating spaces, "empty in the fixed, but often unobserved in the fresh, condition. . . . Each cell has a process at one pole, with an axial wavy channel connected with one of the neighbouring excretory capillaries . . . , the wall of which passes insensibly into the membrane of the cell." Perhaps also certain large amoeboid cells with nuclei filling up almost the whole of the cell and large nucleoli surrounded by clear areas, found by Will in specimens of *C. laticeps* fixed in Flemming's solution and crude acetic acid and described under the nervous system, may rightly belong to this category of peculiar excretory cells.

REPRODUCTIVE ORGANS

On the whole the reproductive organs of this species (Fig. 6) closely resemble those of the species of *Caryophyllaeus*. In the longitudinal direction they extend from 1.5 to 2.5mm. behind the scolex, where the foremost vitelline follicles are situated, to the posterior end of the worm. The openings and the central connections of the ducts are located, however, near the posterior end, the former, in fact, only from 1.5 to 2.8mm. from the tip, depending on the degree of contraction of the specimen. Excepting Skrjabin, the European writers emphasize in their descriptions of the species of *Caryophyllaeus* the fraction of the whole length of the worm occupied by the

organs behind the opening of the cirrus. For *C. tuba* the latter opens at the beginning of the last quarter of the body, for *C. laticeps* at the beginning of the last fifth, and for *C. fennicus* in the last fifth. Skrjabin says only that in *C. syrdarjensis* the ovary is situated in the posterior third of the body. Owing to very considerable differences in degree of contraction and elongation it seems to me that, at least so far as the present species is concerned, these proportions are not of specific value. On account of the greater development of the musculature anteriorly that portion of the body ahead of the genital openings is much more variable in length than that behind the apertures—hence the above measurements for the latter only.

The genital openings are situated in the midline on the ventral surface from 0.5 to 1.0mm. apart. The cirrus-opening is somewhat transversely elongated and about 0.15mm. in diameter. The opening of the female atrium has the form of a shallow, transverse, crescentic groove, about 0.35mm. in width, with its concave side directed anteriorly. Both apertures are so close together in most of the specimens at hand that they are located at the bottom of a common depression; or, the slight depression accommodating the male opening runs insensibly into the crescentic female atrium.

Male genitalia.—The testes (Fig. 11) are not entirely surrounded by the vitelline follicles as in *C. laticeps* and *C. syrdarjensis*. Anteriorly they begin at the same level as do the latter, and posteriorly they extend to the cirrus-sac or in some cases slightly beyond its anterior border. They are irregularly ellipsoidal in shape, and have lengths, widths and depths of from 0.135 to 0.227, 0.100 to 0.145 and 0.127 to 0.181mm., respectively. Their number as determined by direct count and by calculation from the average number in longitudinal and transverse sections varies from 150 to 160. They are especially noteworthy on account of their showing the various stages of spermatogenesis with almost diagrammatic clearness, a fact which was also noted by Monticelli in the case of *C. tuba* and by Skrjabin in his description of *C. syrdarjensis*. Nevertheless in none of the series of sections cut were any spermatozoa seen in any part of the vas deferens, altho the uteri were in the same preparations well filled with eggs. This would seem to indicate that contrary to the usual procedure among cestodes the female genital organs develop before the male organs and that self-fertilization does not take place.

The vas deferens forms a loose and somewhat triangular mass of coils about 0.32, 0.28 and 0.36mm. in length, width and depth, respectively and situated immediately ahead of the cirrus-sac. Just before entering the latter it expands into a muscular vesicula seminalis having a diameter of from 65 to 90μ and a length of about 0.30mm.; but at its beginning it has no seminal reservoir like that attributed to *C. laticeps* by Will. The wall of the duct consists of a lacerated or pseudociliated, syncitial epithelium, provided with widely separated nuclei—excepting in the seminal vesicle where they are fairly numerous—and resting on a basement membrane. The musculature of the vesicle consists of numerous circular fibres with a few oblique fibres distributed among them.

Entering the cirrus-sac anterodorsally with a diameter of 30μ, the vas deferens expands in the dorsal third of the latter to form a sort of secondary, but doubtless only temporary, seminal vesicle averaging 60μ in diameter. After taking several turns it gradually diminishes to about 35μ in the mid-region of the sac and passes insensibly into the cirrus proper. The structure of the wall of the duct within the sac up to this point is the same as that of the seminal vesicle just outside of the sac. The cirrus, which occupies the lower half of the cirrus-pouch, is a comparatively large closely coiled tube with a diameter of 60 to 65μ. Its wall, which is much cleft and folded on account of the length of the organ, is similar in structure to that of the vas deferens, excepting that the number of circular muscular fibres is much greater and that the imperfect epithelium of the latter is replaced (in the transitional region) by smooth cuticula, continuous with that of the ventral surface of the worm as in the cestodes proper. Altho in the material at hand there were no cases of extruded cirrus, its structure and disposition within the sac is such as to lead one to believe that when it is evaginated it is a comparatively long and stout organ.

The cirrus-sac (Fig. 12) is ellipsoidal in shape and occupies the whole of the medulla of the region dorsoventrally and almost all of it laterally. Its length, width and depth are, respectively, 0.40 to 0.50, 0.50 and 0.50 to 0.60mm. Its wall is composed of muscular fibres running in all directions and not sharply separated from the retractor muscles within the organ. A few dorsoventral fibres pass from the top of the sac to the dorsal body-wall and a few from

the equatorial region to the ventral body-wall. The contents of the sac are composed of numerous and very compactly arranged retractor muscles, their myoblastic nuclei and a small amount of parenchymatous tissues.

Female genitalia.—Into the dorsal portion of the female genital atrium, which is about 0.25mm. in depth and lined with a much lacerated continuation of the cuticula from the ventral surface of the worm, the vagina empties slightly to one side of the median line, the other side accomodating the opening of the uterus. From the atrium it passes backward in the median line (Fig. 6) beneath, or at some levels almost surrounded by, the coils of the uterus. Its diameter near the opening varies from 50 to 55μ, but half way along its course this is reduced to 30μ. Thruout its length its wall is composed of a lining of cuticula 5μ in thickness and surrounded by numerous circular muscles only, the myoblastic nuclei of which form a rather distinct stratum about 10μ distant from the fibres. At the level of the posterior end of the ovary it opens into the oviduct with a diameter of 8μ and a much reduced cuticular lining and layer of circular muscles. Unlike that of *C. laticeps*, as described by Will, it is nowhere enlarged to form a receptaculum seminis.

The ovary is situated usually half way between the genital openings and the posterior end of the animal (Fig. 6). It is from 0.8 to 0.9mm. in length and consists of a stout almost spherical isthmus, about 0.4mm. in diameter, from which numerous, irregular and thick lobules pass upward and slightly forward to enclose a capacious generative space. In the latter respect this form resembles not only the species of *Caryophyllaeus* but also *Cyathocephalus* and *Bothrimonus* as described elsewhere by the writer (Cooper, 1919). As shown in figure 13, the lobules lie in the periphery of the medulla, close to the main longitudinal muscles. Ova near the beginning of the oviduct average 15μ in diameter in sections, and are composed almost entirely of the nucleus, there being very little cytoplasm. A distinct and almost spherical nucleolus taking the counterstain very readily is to be seen in each nucleus.

The oviduct begins at the posterior end of the isthmus and somewhat ventrolaterally in an oocapt, 25μ in diameter by 20μ in length and provided with only a few circular muscles. About 125μ from the oocapt it is joined by the vagina. This first portion

of the oviduct is 25 to 30μ in diameter, and takes a dorsal course. Its walls are composed of a thin but uniform layer of circular muscular fibres on the outside, and on the inside of a comparatively thick layer of epithelium, the cells of which are not clearly separated from each other but contain relatively large and deeply staining nuclei. After passing backward and upward about 40μ beyond the point of union with the vagina the oviduct receives the common vitelline duct.

As in the species of *Caryophyllaeus* the vitelline follicles are located in the medulla in two distinct and separate regions: a large one extending from 1.5 to 2.5mm. behind the tip of the scolex to the cirrus sac, and a much smaller one in the more or less conical posterior end of the worm behind the coils of the uterus (Fig. 6). In the former situation they form an irregular layer in the periphery of the medulla (Fig. 11), for not only do some dip down among the testes, as mentioned above, but others extend outward to the main longitudinal muscles; in the latter, however, they occupy almost the whole of the medulla, as in *C. laticeps*. In the immature worm there is, furthermore, some tendency for them to be arranged in two lateral fields anteriorly, leaving a free strip in the median line dorsally and ventrally. In the anterior region in particular they are very numerous, irregularly ellipsoidal in shape, and vary greatly in size. From 8 to 14 appear in transections, while their maximum diameter is 0.20mm. Posteriorly they are slightly larger.

The process of the formation of the peculiarly clear yolk-cells which are to be seen in the vitelline ducts (Fig. 14c) can be followed with a considerable degree of satisfaction in the follicles. The cytoplasm of the small peripheral primordial cells from which they develop is very compact, and consequently stains deeply as does the nucleus (Fig. 14a). Numerous vacuoles appear in it and quickly enlarge, so that in the intermediate stages the nucleus appears to be suspended in the centre of the cells by protoplasmic strands radiating from it to the cell-membrane, as shown in figure 14b. These strands become modified into numerous, spherical deutoplasmic granules, migrate outward and eventually come to lie just inside the cell-membrane (Fig. 14c). In the proximal part of the uterus, where from four to six vitelline cells are seen to be associated with each fertilized ovum in the formation of the egg, the nucleus enlarges still

more and becomes more transparent, while the cell-wall gradually breaks down, thus liberating the vitelline granules. The enlarged nuclei remain intact, however, during the passage of the egg thru almost the whole length of the uterus.

The common vitelline duct varies in diameter from 30 to 75μ, and is lined by an epithelium similar to that of the oviduct. It is largest immediately dorsal to the posterior end of the ovarian isthmus where it forms a vitelline reservoir, as in *C. laticeps*, as much as 220μ in width by 45μ in depth when filled with yolk. A little farther forward it receives two main tributaries, varying considerably in calibre according to the amount of vitelline material they contain. Whereas these two ducts collect chiefly from the follicles ahead of the uterus, at least one small tributary on each side drains the follicles situated in the posterior end of the worm, and unites with the main ducts near their point of union with each other.

Shortly after being joined by the common vitelline duct and as it courses a little farther back on one side or the other, the oviduct becomes surrounded by a poorly developed shell-gland. The ootype is consequently inconspicuous. Beyond the ootype the epithelium is syncitial in its nature since no distict cell-boundaries appear. More than its inner half is deeply cleft to form pseudocilia, yet its nuclei are comparatively large. As the oviduct—now, more properly called the beginning of the uterus—continues backward in a dorsal position in the medulla, it gradually enlarges, according as it becomes filled with eggs, its wall becomes thinner and thinner, and the nuclei diminish in number, flatten out and eventually disappear. The latter takes place particularly after the organ turns in its course—just ahead of the posterior group of vitelline follicles—and starts forward towards the female genital atrium.

From a point just behind the level of the posterior border of the ovarian isthmus to its opening the uterus is surrounded by a voluminous mass of club-shaped, unicellular glands (Fig. 13), similar to those described for the species of *Caryophyllaeus* and closely resembling those described by the writer (1919) for *Cyathocephalus americanus* and *Bothrimonus intermedius*. As to the function of these cells no definite statements can be made as yet. Monticelli likened the similar cells in *C. tuba* to those to be seen along the uteri of many trematodes as well as of *Gyrocotyle urna* (Wagener), and called them

glutin-producing glands. Will described them in *C. laticeps*, and said that they were "fully identical" with those in *Diphyllobothrium latum*. He also incidentally mentioned that Saint-Remy (1890) looked upon them as a shell-gland. Schneider (1902) called them glandular cells in *C. fennicus*, while Skrjabin considered them to be shell-glands in *C. syrdarjensis*. In view of the fact that, as in the species of the subfamily Cyathocephalinae just mentioned, the shell-gland surrounding the ootype is poorly developed—altho it was clearly seen in this species to initiate the formation of the egg-shell—they may act as an accessory shell-gland. Even tho this whole region of the uterus is lined with a deeply cleft cuticula, numerous droplets of material were seen in the sections studied adhering to or lying among the pseudocilia as if they were secreted from the cells in question; and it is only in this portion of the uterus, not in the thin-walled proximal region, that the shells of the eggs are thickest. At any rate, since the uterus is provided with only a very few scattered circular muscles, excepting just before its opening, they cannot be myoblastic in their nature. Distally they diminish considerably in number, yet they are directly continuous with the myoblastic nuclei of the more numerous muscular fibres surrounding the terminal portion of the duct and the female atrium, which in turn are continuous with the subcuticular cells around the atrial opening. As stated above, the uterus opens into the female genital atrium ahead of and slightly to one side of the vagina. The atrium itself is from 0.20 to 0.30mm. in length by about 0.10mm. in diameter and lined with a very irregular and deeply cleft cuticula.

The mature fresh eggs, when examined in normal saline solution, were found to be ovoid in shape and from 54 to 66μ in length by 38 to 48μ in width. The shell is from 2 to 3μ in thickness, and is provided at its larger end with a small button-like boss and at its smaller end with an operculum from 12 to 16μ in diameter.

LIFE HISTORY

As regards the development and life-history of this species only a few statements can be made at present. Larvae as small as that shown in figure 15 were found in the stomach of the host, but, altho a thoro dissection of the food-contents, which consisted of larvae of *Chironomus* and *Simulium*, Ostracoda, Cladocera, "caddice-worms,"

dragon-fly nymphs and Mollusca, was made, their mode of entrance was not discovered. Possibly further search will show that some member of these groups of animals, if not a tubificid worm as in Europe, is the intermediate host. Finally, from the standpoint of the systematic position of the species it should be emphasized that the smallest larvae found had nothing whatsoever in the nature of appendages.

SYSTEMATIC POSITION

From the above description it is clear that this species, altho a member of the family Caryophyllaeidae Lühe 1910, does not belong either to *Archigetes* or to *Caryophyllaeus*. As pointed out above, the scolex resembles that of at least one species of *Archigetes*, namely, *A. brachyurus* Mràzek, but is quite different from the simple, leaf-like anterior ends of the species of *Caryophyllaeus*. The reproductive organs, it is true, are much more comparable to those of the latter, but certain features of the muscular, excretory and nervous systems do not permit of its being placed in either genus. Consequently a new genus is erected to accommodate this form, and is given the following characters:

Glaridacris gen. nov.

With the characters of the family. Medium sized caryophyllaeids with the anterior end modified to form a scolex, provided on each surface with three suckers, of which the median one is the deepest and most efficacious. Main longitudinal parenchymatous muscles in eight large fasciculi in the anterior part of the neck and the base of the scolex. Only two main nerve strands in the medulla, connected in the scolex by two more or less diffuse commissural loops. Excretory vessels form a single cortical plexus with eight principal longitudinal channels; no true flame-cells present, terminal renal organs, peculiar, highly vacuolated, simple cells. Expansion of the vas deferens before entering the cirrus-sac to form a vesicula seminalis. δλαρίς, chisel; ἄκρις, summit.

Type, and as yet only, species: *G. catostomi* sp. nov.

The principal specific characters may be set down as follows:

Glaridacris catostomi sp. nov.

With the characters of the genus. Small cestodarians, up to 25mm. in length by 1.0mm. in breadth. Scolex, short and broad, chisel-shaped in older specimens, hexagonally pyramidal with prominent terminal disc in younger, base large in both; length, 0.30 to 0.45mm., width (posteriorly), 0.45 to 1.10mm., depth (posteriorly), 0.50 to

0.75mm. Neck only slightly narrower than body, 1.5 to 2.5mm. in length; whole worm, apart from scolex, cylindrical, with somewhat conical posterior end.

Cuticula, 7 to 11μ in thickness; subcuticula, 90 to 100μ. No "fibrous strands" nor calcareous bodies in parenchyma.

Female genital atrium, 0.5 to 1.0mm. behind opening of cirrus, 0.20 to 0.30mm. in depth by 0.10mm. in diameter, opening crescentic, in same depression with male opening.

Testes not completely surrounded by vitelline follicles; extend to cirrus-sac posteriorly; irregularly ellipsoidal in shape, from 0.10 to 0.18mm. in different diameters; 150 to 160 in number. Vas deferens, a loose somewhat triangular mass ahead of cirrus-sac, 0.28 to 0.36mm. in diameter. Vesicula seminalis, 0.30 by 0.06 to 0.09mm. Cirrus-sac large, almost spherical, occupying almost whole of medulla of region, 0.40 to 0.60mm. in diameter. Cirrus, 60 to 65μ in diameter.

Vagina median, ventral, 30 to 55μ in diameter. Ovary irregularly lobular, 0.8 to 0.9mm. in length, with nearly spherical isthmus, 0.4mm. in diameter. Oocapt, 20 by 25μ. Vitelline follicles not completely surrounding the testes, 8 to 14 in transections, 0.20mm. in maximum diameter. Vitelline reservoir, the expanded common vitelline duct, 220 by 45μ. Ootype inconspicuous. Uterus in two portions, a proximal, thin-walled, and a distal, extending from the posterior vitelline follicles to the opening and surrounded by a large number of unicellular glands; empties into female atrium slightly ahead of and to one side of vagina.

Eggs, ovoid, with small boss at larger end, 54 to 66μ in length by 38 to 48μ in width.

Habitat: In stomach and intestine of *Catostomus commersonii* (Lacépède).

Finally, Lühe's (1910) characterization of the family will have to be slightly emended to include this new species:

CARYOPHYLLAEIDAE Lühe 1910, e.p.

Monozootic pseudophyllidea with scolex unarmed; may or may not bear more or less well expressed sucking organs which are set off from the rest of the body by a neck-like constriction or are fused with the same without such. A caudal appendage bearing on its hinder end the hooks of the oncosphere may also be present in the sexually mature animal. Genital organs present only singly. Reproductive openings surficial, ventral, medial and near the posterior end. Testes, numerous, exclusively anterior to the ovary and the female genital ducts. Cirrus unarmed, ahead of the female sexual apertures; vagina and uterus open at the bottom of a common vestibule which resembles in its histological structure the shallow genital atrium and opens into it close behind the cirrus. Ovary two-winged, directly behind the genital opening. Vitelline follicles in the medulla, but peripheral to the testes and more or less completely surrounding them like a mantle; mostly ahead of the ovary, but a group also in the hinder end of the body, separated from the main mass by the ovary and the female genital ducts. Uterus a winding canal, without sack-like expansions. Eggs, operculate.

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EXPLANATIONS OF FIGURES

co cirrus opening
cs cirrus-sac
ev excretory vessel
fa female atrium
g glands
i isthmus of ovary
lm longitudinal muscles
n nerve(s)
ns nerve strand

o	ovary	*t*	testis
olm	outer longitudinal muscles	*u*	uterus
rc	renal cell	*v*	vagina
sg	shell-gland	*vf*	vitelline follisicles
		vs	vesicula seminalis

Unless otherwise stated, the lines indicating the magnifications of the figures are 0.5mm. in length.

PLATE I

Fig. 1. Surficial view of scolex of specimen 3.5mm. in length.
Fig. 2. Lateral view of same.
Fig. 3. Surficial view of scolex of specimen 21mm. in length.
Fig. 4. Lateral view of same.
Fig. 5. Scolex of *Archigetes brachyurus*, surficial view. After Mrázek.
Fig. 6. Genital organs in posterior end of worm, toto preparation, surficial view.
Fig. 7. Pits in the mucosa of the host's intestine, each showing only two of the several larvae found in them.
Fig. 8. A terminal renal cell and its connections, from a frontal section. The line at the side represents 0.05mm.

PLATE II

Fig. 9. Transection thru the middle of the scolex.
Fig. 10. Transection thru the anterior part of the neck.
Fig. 11. Transection thru about the middle of the whole worm.
Fig. 12. Transection thru the cirrus-sac.
Fig. 13. Transection thru the ovarian isthmus.
Fig. 14. Three stages in the development of the vitelline cells: *a*, the primordial cell from the periphery of the follicle; *b*, an intermediate stage from the centre of the follicle; *c*, the mature cell from the vitelline reservoir. The line repersents 0.02mm.
Fig. 15. The smallest larvae procured.

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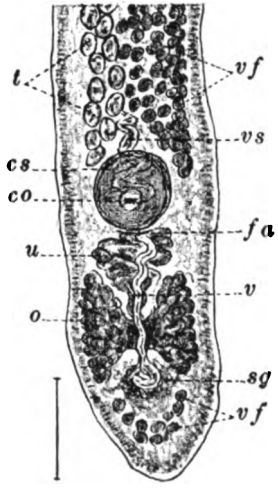

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PLATE I

COOPER

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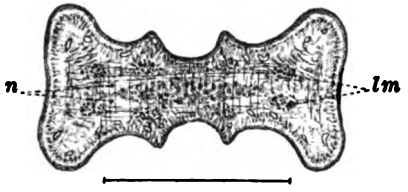

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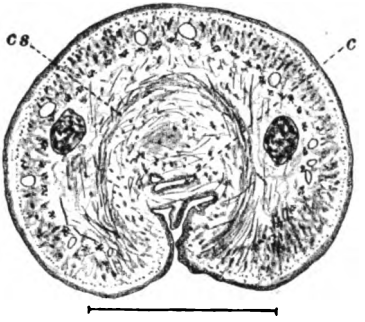

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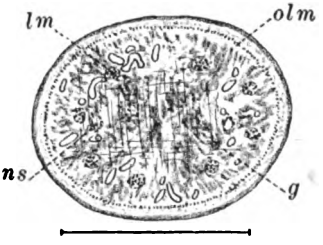

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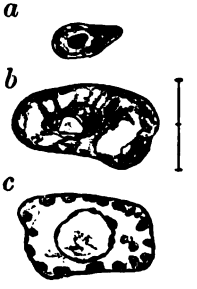

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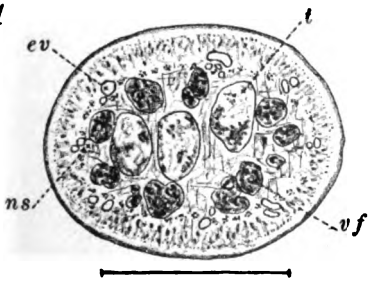

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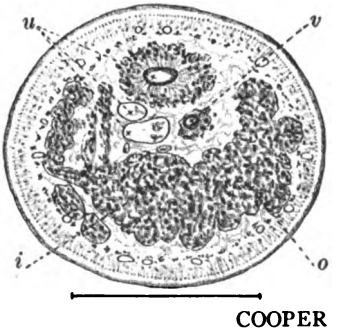

PLATE II

COOPER

THE GENERA OF THE ENCHYTRAEIDAE (OLIGOCHAETA)[1]

By Paul S. Welch

INTRODUCTION

Michaelsen's monograph (1900) on the Oligochaeta contains the last general revision of the genera of the Enchytraeidae for the whole world. Eisen (1905) modified, to some extent, the genera then known to occur in North America. Since the publication of the above-mentioned works numerous contributions to the knowledge of these annelids have been made, so that the family has grown from a relatively small group containing 13 genera and less than 100 species to the present status of 16 genera and approximately 325 species. With this marked increase has come the necessity for certain changes and modifications in the limits of most of the groups.

The revision herein presented is the direct result of the discovery of certain enchytraeids which failed to agree exactly with any of the older generic descriptions and in order to properly assign them a careful survey of several genera was necessitated. It was then decided to extend the study to include all of the known genera of Enchytraeidae and thus not only make available a considerable amount of inaccessible material but also present something which will serve as a basis for further revision as soon as more data are secured. The writer wishes to acknowledge indebtedness to one of his graduate students, Miss Helen M. Scott, who gave considerable assistance in testing and rechecking the revised generic descriptions.

This revision can at best be regarded only as an attempt to indicate progress to date. Certain unsurmountable difficulties make it impossible at the present time to do more than work over critically the published records as they now stand and to determine the present status of each group as nearly as possible. The descriptions of all of the species now assigned to the Enchytraeidae (approximately 325) have been re-examined in this connection and the work of the writer on this group of annelids, covering a period of ten years, has been brought to bear upon the task wherever possible. The

[1] Contribution from the Zoological Laboratory of the University of Michigan.

principal difficulties are indicated below in order to point out some of the features which should receive attention in future investigations and revisions:

1. Descriptions based upon sexually immature specimens or upon material not so stated but apparently immature.

2. Species placed tentatively in certain genera but data insufficient to make final disposal of them.

3. Lack of information on morphological features now known to have important systematic value, especially in the older descriptions

4. Deviations which strongly have the appearance of being errors of observation or of printing.

5. Structures recorded as "not seen" or "not found" sometimes the result of faulty methods of study, such as external examination only or dissection only, or the use of poorly preserved specimens.

6. Difficulty in correct interpretation of certain descriptive terms, as for example, does "lobulated testes" mean *divided* testes as represented in *Lumbricillus* or something much less significant. In the absence of illustrations which supplement the descriptions, such expressions are very puzzling.

7. Interpretation of indefinite terms indicating differences of degree, as for example, "setae slightly curved," an expression which when unaccompanied by further explanation or by figures is practically unusable.

8. Difficulty arising from descriptions which fail to mention important organs. Does lack of mention always or ever mean positive absence of the structure? Apparently, many investigators have not realized the importance of stating *positively* that certain characters are absent. To leave these matters without mention is a distinct detriment.

In this revision, the generic descriptions have been so modified as to include what seems to be well founded changes demanded by increased data as well as new features now regarded as having generic value. In making these modifications the procedure has been as follows:

1. The writer concurs with those who hold that the multiplication of genera should be avoided except where the case is perfectly clear.

2. Genera and species founded upon sexually immature material have been disregarded. It is well established that complete, depend-

able data cannot be secured from immature specimens and it is to be hoped that future investigation will frown upon any disposition to write descriptions from such imperfect material. It can only lead to confusion and hinderance.

3. Data easily derivable from illustrations not supplemented by description were regarded as valid.

4. Lymphocytes and the brain have been omitted purposely from generic consideration since the writer doubts their value in generic distinction.

5. In certain cases, statements known to be true for some of the species of a genus have been incorporated in the generic description since they represent all that is known at present about the features mentioned. Re-examination of the other species will decide whether such features will remain valid.

6. When the usual generic characters are not mentioned in the descriptions, such omission is taken to mean that no information is available, rather than that they are absent.

7. There have been incorporated into the genus descriptions certain features which may prove later to be specific characters, as for example, when structures are indicated as "present and absent." This is done largely for the sake of record and to indicate divergences from the former descriptions. It remains for future investigation to make the final disposal.

8. The term *chylus cell* is used to indicate the large, intestinal cells each of which is characterized by a longitudinal, intracellular canal. In the region involved, chylus cells usually alternate with the ordinary epithelial cells which line the lumen of the intestine.

9. Eisen's system of classifying the various forms of penial bulb has been followed to considerable extent. There is increasing evidence that the features of the penial bulb have distinct generic value.

10. No attempt has been made to list more than the most important literature involved in making this review. A complete set of references and a statement of the synonymy up to 1900 is given in Michaelsen's monograph.

Subfamilies

Some attempts have been made in the past to establish subfamilies in the Enchytraeidae. Eisen (1905, pp. 11–13) proposed

four subfamilies, *Mesenchytraeinae*, *Enchytraeinae*, *Achaetinae*, and *Lumbricillinae*, the major basis of distinction being the character of the penial bulb. However, since the structure of the penial bulb was not known for certain genera, the distribution of the genera among these subfamilies was to some extent inferential. Čejka (1910, p. 25) made use of three subfamilies under the names *Fridericiinae*, *Mesenchytraeinae* and *Henleinae*.

There seem to be some good grounds for considering the structure of the penial bulb as a basis for the erection of subfamilies, but since its structure is unknown in such genera as *Achaeta*, *Distichopus*, *Chirodrilus*, and *Stercutus*, it does not seem profitable just now to attempt to discuss this problem.

The Penial Bulb

The first attempt to use the characters of the penial bulb in the classification of the Enchytraeidae was made by Eisen (1905, pp. 6–10) who, after an extensive study of a large number of North American species, thought it possible to recognize three distinct "types" which were definitely related to certain taxonomic groups. The writer (Welch, 1914, pp. 173–180) presented a critical discussion of this matter, pointing out that it seemed necessary to make some modifications in Eisen's original system. Since that time many more of the North American forms have been studied and while it is still probable that certain changes may ultimately be necessary, all of the evidence at hand indicates that the characters of the penial bulb are valuable in generic and possibly in specific diagnoses. For this reason, statements as to the penial bulb have been incorporated into revised definitions of the various genera, retaining Eisen's terms for the different types. If many of the species from the Old World can be re-examined and the structure of the penial bulb described and figured, the taxonomic status of this organ will be made more certain.

For sake of ready reference, Eisen's summary of the three types of penial bulbs will be quoted here.

"The Mesenchytræid bulb is a single muscular structure, containing circular muscles as well as fan-shaped muscular bands connecting the body wall with the periphery of the bulb. Between the muscular bands are generally found numerous penial glands which

open on the surface of the bulb around the penial pore. The sperma-duct penetrates the bulb, opening on the center of its outer surface.

The Enchytræid bulb is multiple, consisting of several separate cushions grouped around the penial pore. In these cushions we find several sets or fascicles of glands, each fascicle opening by itself on the surface of the body. There are no muscular bands connecting the base of the cushions with its periphery. The sperm-duct never penetrates the bulbs or cushions but opens close to and independently of them. Exterior to the cushions there are numerous muscles connecting the body wall immediately surrounding the pore with other parts of the same somite.

The Lumbricillid bulb is always single and covered with a strong muscular layer, which however never penetrates down between the cells of the bulb. There are generally two or three distinct sets of glandular cells in the bulb. Some of these open in the lower part of the sperm-duct, or rather in a narrow groove in the elongation of the sperm duct. Others open on the free surface of the bulb, either irregularly or in narrow circular fields, bunched into fascicles. The sperm-duct penetrates one side of the bulb. In *Bryodrilus* the gland which opens in the extension of the sperm-duct is covered with a thin cushion of muscular strands, forming a bulb within a bulb."

Relationships

It is not intended that any particular significance be attached to the order in which the different genera are treated in this paper. Certain genera are too poorly known at present to justify any attempt to establish relationships, while others are little enough known to make it a difficult and an uncertain task. It thus seems best in this paper to omit efforts to determine phylogeny.

PROPAPPUS MICHAELSEN

Setae sigmoid; distal extremity cleft; those of a bundle equal; four bundles per somite, two lateral and two ventral. Dorsal pores absent. Oesophagus passing abruptly into intestine in 8; intestinal diverticula absent; chylus cells absent; peptonephridia absent. Origin of dorsal blood-vessel anteclitellar or intraclitellar. Nephridia with small, slender, funnel-shaped anteseptal part and with loose, scantily lobed, irregularly folded postseptal body, the folds being but

little more than in close contact. Testes undivided; moderately compact. Spermiducal funnel extremely short; shallow bowl-shaped. Sperm duct very short; confined to 12. Penial bulb absent; small atrial chamber at ectal end of sperm duct; atrial glands absent. Spermathecae simple; no diverticula; no connection with digestive tract; long, extending into 6–12.

Discussion

Formerly, the genus *Henlea* (Michaelsen, 1903, p. 51) was regarded as the most primitive group of the Enchytraeidae because of the diverse character of the setae manifested by the various species although it presented no distinct transition features leading into the near-standing, more primitive families of Oligochaeta (Phreodrilidae, Tubificidae, Naididae). In 1905, however, Michaelsen (pp. 24–28) described under the name *Propappus* a genus based upon specimens found abundant in Lake Baikal, Southern Siberia, at depths of 2–8 meters. These specimens presented a complex of characters of particular interest. Most of the fundamental features are enchytraeid leaving little doubt as to its membership in that group. However, certain affinities with other families are manifested in the presence of the following structures:

1. Cleft setae are recorded for the first time among the Enchytraeidae, all other known species having the simple-pointed type. Cleft setae are common in Naididae, Tubificidae, and Lumbriculidae.

2. The spermiducal funnel is a very short, shallow, bowl-shaped organ, resembling the funnel in certain other oligochaetes (*Tubifex*, et al) and showing little resemblance to the elongate, cylindrical, glandular, thick-walled funnel found in practically all enchytraeids. Apparently, only two other enchytraeids have spermiducal funnels which at all resemble those of *Propappus*, namely, *Mesenchytraeus bungei* Mchlsn. and *Mesenchytraeus grebnizkyi* Mchlsn. (Michaelsen, 1901, pp. 193, 199), in which they are very short, and "pantoffelförmig."

3. Each nephridium consists of a small, slender, funnel-shaped nephrostome which constitutes the entire anteseptal part. The postseptal part, however, departs strikingly in form and structure from the typical enchytraeid condition in being very loosely constructed, having the appearance of an irregular knot of adherent loops

or folds, the free end of which composes the efferent duct. This type of nephridium recalls the postseptal coils in the same organ in Tubificidae, et al. Of all the other enchytraeids, *Mesenchytraeus* alone shows any approach to such nephridial structure, although its irregular, lobed, postseptal part in which the wide ducts are close together is definitely coalesced into one mass.

Only two species are known at present to belong to this annectent genus, namely, *glandulosus* and *volki*. The former, found in Lake Baikal, in the one on which the genus was established. Recently, Michaelsen (in a paper dated 1915 but which must have been published in 1916 since papers dated 1916 are referred to in it) described a second species, *volki*. It appears that this same writer first reported it in the "Hamburger Nachrichten, Jahrg. 1916, Nr. 53, vom 30, Januar, 3. Beilage, p. 1," as *Palpenchytraeus volki*, n. gen., n. sp. but later placed it in *Propappus*—a decision which certainly seems more nearly correct. It is worthy of mention that in this species the elongated spermathecae recall the condition in many of the North American mesenchytraeids.

HENLEA MICHAELSEN

Setae straight and unequal in size, or straight and equal in size, or slightly sigmoid and approximately equal in size; distal extremities simple-pointed; four bundles per somite, two lateral and two ventral. Head pore at 0/1. Dorsal pores absent. Oesophagus (with possible rare exceptions) expanding abruptly into intestine. Peptonephridia present or absent. Intestinal diverticula usually present. Origin of dorsal blood-vessel anteclitellar; rarely intraclitellar; cardiac body absent. Blood colorless. Nephridia with either large or small anteseptal part; nephridial canal loosely wound and surrounded by considerable amount of cell mass. Ventral glands absent. Testes compact; not divided. Spermiducal funnel cylindrical; sperm duct short, confined to 12, rarely longer. Sperm sacs and ovisacs absent. Penial bulb of lumbricillid type. Spermathecae connecting with digestive tract; diverticula present or absent.

Discussion

Perhaps no genus of Enchytraeidae needs a thorough going revision as badly as does *Henlea*. Its heterogeneous nature has been

recognized by investigators for some time but certain conditions surrounding the problem have thus far made such a revision almost impossible. It therefore presents many difficulties in connection with the present attempt to redefine the genus. Friend (1914b, pp. 150–153; 1915, pp. 197–198) has pointed out the existence of certain "groups" within this genus. The writer [Welch] suspects strongly that *Hepatogaster* Čejka should be regarded as a part of the genus *Henlea*—possibly as a subgenus. It seems likely that these "groups" will form the basis for the establishment of several subgenera when the genus is thoroughly worked over, particularly when many of the foreign species have been re-examined and more thoroughly described.

Certain deviations, apparent or otherwise, from the newly modified genus definition require some notice. Some ill-defined species (*lefroyi* Beddard; *scharfii* Southern; et al) seem to offer exceptional features, but the imperfect descriptions leave considerable doubt as to whether they belong in this genus at all. Hence no significance can be attached to them at present.

Eisen (1905, p. 98), in connection with his discussion of *Henlea*, presents the following statement: "Chylus cells in the intestine in the vicinity of clitellum." However, in his subsequent descriptions, no mention of them appears except in the case of *H. guatemalae* (pp. 102–103) which is described as having no chylus cells at all. In none of the American species of *Henlea* examined by the writer have chylus cells been observed.

A number of species have been described in which the origin of the dorsal blood-vessel is specified as intraclitellar and the definition has been modified so as to include these forms. However, Friend (1913b, pp. 460–461) has described a species under the name *insulae* which has the dorsal blood-vessel arising in "17/18 or 19/20." This form is assigned to *Henlea* but taking the original description as it stands, the writer is unable to place it with any more certainty in *Henlea* than in one or two other genera, as for example, *Enchytraeus*. For this reason, the apparent exception has not been given any particular consideration. *H. alba* (Friend, 1913c, p. 83) and *H. hillmani* (Friend, 1914b, p. 135) are reported as having the origin of the dorsal blood-vessel in the region of 13–14.

Of the sixty or more species now assigned to this genus, there are several which future investigations will certainly prove invalid.

HEPATOGASTER ČEJKA

Setae straight and equal; distal extremities simple-pointed; four bundles per somite, two lateral and two ventral. Head pore at 0/1. Dorsal pores absent. Oesophagus merging gradually into intestine. Peptonephridia present, dorsal and ventral. Intestinal diverticulum present, surrounding digestive tract. Chylus cells absent. Origin of dorsal blood-vessel anteclitellar; cardiac body absent. Nephridia with small anteseptal part; nephridial duct loosely coiled and with distinct cell mass. Testes not divided. Spermiducal funnel cylindrical. Penial bulb of lumbricillid type. Spermathecae connecting with digestive tract; diverticula absent. Characteristic, longitudinal canals in epithelium of digestive tract in posterior part of body just entad of perivisceral blood-sinus.

Discussion

The genus Hepatogaster was established by Čejka (1910) for the reception of two species which he considered as presenting characters representing a new group. A careful examination of descriptions reveals at least a close affinity with *Henlea*. In fact, it could be included in *Henlea* with practically no change in the limitations of the latter. Only one feature seems to offer any difference, namely, that the oesophagus passes gradually into the intestine, but it seems doubtful if a new genus could be established upon that character alone. The presence of certain peculiar longitudinal canals in the epithelium of the posterior part of the alimentary canal is stressed in the original description and while these characters seem to be unique, their value as a generic character remains to be demonstrated.

The structure of the penial bulb requires some notice. Čejka thought that it resembled the enchytraeid type, interpreting certain peculiar glands which open out through the body-wall in 12 and 13 in the vicinity of the sperm duct termination as parts of the penial bulb proper. Unfortunately, the penial apparatus is recorded in only one of the two species. However, a careful study of the description and Čejka's plates leads the present writer to hold that the bulb is of the lumbricillid type for the following reasons: 1. The sperm duct opens

to the exterior through a compact, glandular bulb which is typically lumbricillid. This duct actually opens out through it into a penial invagination—a thing which does not occur in the typical enchytraeid bulb. (2) Of the nearby groups of problematical glands, the one in 12 is single and median, thus apparently belonging to neither bulb. (3) The other glands are in 13—another somite—a thing which has not been observed in connection with the various parts of a typical enchytraeid bulb. (4) In general appearance these peculiar glands resemble the "ventral glands" found in certain enchytraeids although they are unusual in being free from direct connections with the ventral nerve cord.

Owing to the incompleteness of some of the data on this proposed genus, it is allowed to stand for the present although there seem to be good reasons for believing that it should be reduced at least to the rank of a subgenus of *Henlea.*

BRYODRILUS UDE

Setae slightly or distinctly sigmoid; distal extremities simple-pointed; those of a bundle equal in size; four bundles per somite, two lateral and two ventral. Head pore at 0/1. Dorsal pores absent. Oesophagus merging gradually into intestine. Peptonephridia present. Four intestinal diverticula present. Origin of dorsal blood-vessel intraclitellar; cardiac body present or absent. Nephridia with small anteseptal part; nephridial canal loosely wound; cell mass large. No ventral glands. Testes compact; not divided. Spermiducal funnel cylindrical; sperm duct confined to 12. Sperm sacs and ovisacs absent. Penial bulb of lumbricillid type. Spermathecae connecting with digestive tract; diverticula absent.

Discussion

While a few slight modifications have been introduced into the description of this genus, no important comments are demanded here. No mention of intestinal diverticula appears in the description of *B. sulphureus* (Bretscher, 1904, p. 262) but since the material on which the description was based was immature, this omission may have no significance. The head pore in this same species is recorded as appearing on the tip of the prostomium.

Four species are assigned to this genus.

BUCHHOLZIA MICHAELSEN

Setae sigmoid; distal extremities simple-pointed; those of a bundle approximately equal in size; four bundles per somite, two lateral and two ventral. Head pore at 0/1. Dorsal pores absent. Oesophagus expanding abruptly into intestine. Peptonephridia present. Chylus cells absent. Origin of dorsal blood-vessel anteclitellar or intraclitellar; arising from summit of dorsal intestinal diverticulum; cardiac body absent. Blood colorless. Nephridia with anteseptal part large or small. Spermiducal funnel cylindrical; sperm duct confined to 12. Structure of penial bulb unknown. Spermathecae connecting with digestive tract; diverticula absent.

Discussion

In *Buchholzia focale* (Friend, 1914a, pp. 118–119) no mention is made of a dorsal intestinal diverticulum and the origin of the dorsal blood-vessel is given as "Henlean."

But little is known concerning the penial bulb in representatives of this genus. Eisen (1905, p. 12) places the genus under his subfamily Lumbricillinae but explains (p. 6) that he does so on account of its "undoubted relationship to the genus *Henlea*."

Buchholzia parva (Bretscher, 1900a, p. 24) is described as showing no connection of the spermathecae with the digestive tract. However, the sexual maturity of the material might be questioned since it is stated that no trace of a clitellum was found.

Six species are now assigned to this genus.

MARIONINA MICHAELSEN

Setae sigmoid; distal extremities simple-pointed; those of bundle approximately equal in size; four bundles per somite, two lateral and two ventral. Head pore at 0/1. Dorsal pores absent. Oesophagus merging gradually into intestine. Peptonephridia absent. Intestinal diverticula absent. Chylus cells absent. Origin of dorsal blood-vessel postclitellar; cardiac body absent. Blood red, yellow, or colorless. Nephridia with anteseptal part large or small; nephridial canal loosely wound; cell mass large. Ventral glands present or absent. Testes undivided. Spermiducal funnel cylindrical; sperm duct confined to 12. Sperm sacs present or absent. Penial bulb of the lumbri-

cillid type. Spermathecae with or without connection with digestive tract; never greatly elongate; diverticula present or absent.

Discussion

In a few species, assuming that they are correctly referred to this genus, there seems to be some deviation as to the origin of the dorsal blood-vessel. Bretscher (1900b, p. 449; 1901, pp. 209–10) described *rivularis* and *guttulata* as having this origin anteclitellar, and Eisen (1905, p. 91) reported it in 12 in the single specimen of *alaskae* which he described although he retained the general generic character of a postclitellar origin (p. 90).

Friend (1912a, p. 224) has described a species, *sialona*, which he assigns to *Marionina*, pointing out at the same time that it is strikingly like an *Enchytraeus*. This species possesses peptonephridia—a feature not represented in *Marionina* and *sialona* is unique in that respect if it actually belongs in *Marionina*. However, the writer has been unable, on the basis of the original description, to see why that species should not be assigned to *Enchytraeus*, rather than to *Marionina*. If this be the proper disposal of *sialona*, then the absence of peptonephridia still stands as an invariable character of the genus.

Eisen (1905, p. 90) held that a generic character appears in the presence of a small sperm sac in connection with each testis. Whether this is true, remains to be determined by future investigations.

In *antipodum* (Benham 1904b, p. 294) the body of the penial bulb appears to be of the lumbricillid type, but it is unique in possessing a single, large accessory gland. Bretscher (1901, p. 210) recorded *guttulata* as "ohne Prostata," but the whole description is so brief that it is impossible to judge accurately as to the sexual maturity of the material studied, or as to the exact meaning of the above quoted statement.

M. werthi Mchlsn. (1908, p. 15) has a penial bulb which is described as "einen winzigen, zwiebelförmigen, ganz in der Leibeswand verborgenen Bulbus aus. An diesen Bulbus, der manchmal als winzige äussere Papille etwas heraustritt, sitzt eine schwach gelappte, in die Leibeshöhle hineinragende Prostata."

About twenty-eight species are referred to this genus.

LUMBRICILLUS ÖRSTED

Setae sigmoid; distal extremities simple-pointed; those of a bundle approximately equal in size; four bundles per somite, two lateral and two ventral. Head pore at 0/1. Dorsal pores absent. Oesophagus merging gradually into intestine. Peptonephridia absent. Intestinal diverticula absent. Chylus cells absent. Origin of dorsal blood-vessel postclitellar, rarely intraclitellar; cardiac body absent. Color of blood yellow, red, or colorless. Nephridia with anteseptal part either large or small; nephridial canal loosely wound, and considerable cell mass between the folds. Ventral glands present or absent. Testes divided deeply, forming a number of distinct lobes. Spermiducal funnel cylindrical; sperm duct long but confined chiefly to 12. Sperm sacs and ovisac absent. Penial bulb of lumbricillid type. Spermathecae connecting with digestive tract; diverticula absent.

Discussion

While the limits of this genus have been but little changed, certain variations may well be mentioned here. *L. viridis* (Stephenson, 1911, p. 48) has the dorsal blood-vessel arising in 13 and in *tuba* (p. 43) it arises in 13, 14, 15. A variety (?) of *minutus* (Müll.) described by Michaelsen (1911, pp. 1–4) has this vessel arising in 12. *Lineatus* (Müll.) (=*agilis* Moore) has also been described by some authors as having the dorsal vessel arising in 13.

Ventral glands do not appear in all representatives of *Lumbricillus*. Furthermore, they are said to occur in a few species of certain other genera (Welch, 1914, p. 141).

Distinct sperm sacs and ovisacs appear to be absent in this genus. A few references to very diminutive ovisacs restricted to the clitellar region occur in the literature (Eisen, 1905, p. 77; Moore, 1905, p. 397) but these can scarcely be regarded as having any special significance. Eisen (1905, pp. 75–76) stated that each division of the testes is capped by a small sperm sac and evidently regarded this as a generic character.

Stephenson (1911) has pointed out the close relation of *Lumbricillus* to *Enchytraeus* on the basis of the discovery of certain species which though assigned to the former, possess some characters strongly suggestive of the latter.

About thirty species are assigned to this genus at present, although there is reason for doubting the validity of some of them.

FRIDERICIA MICHAELSEN

Setae straight or nearly so; unequal, those in bundle developed in pairs, outer pair being largest, and enclosing smaller pairs; distal extremities simple-pointed; four bundles per somite, two lateral and two ventral. Head pore at 0/1. Dorsal pores present. Oesophagus merging gradually into intestine. Peptonphridia present. Intestinal diverticula absent. Chylus cells present. Origin of dorsal blood-vessel postclitellar or intraclitellar, usually the former; cardiac body absent. Blood colorless. Nephridia usually with large anteseptal part, always consisting of more than nephrostome; cell mass well developed. Ventral glands usually absent. Testes not divided. Spermiducal funnel cylindrical; sperm duct short, usually confined to 12. Sperm sacs and ovisacs absent. Penial bulb of lumbricillid type. Spermathecae usually connecting with digestive tract; diverticula present or absent.

Discussion

While it seems advisable to make but little modification in the description of *Fridericia*, a few variations as recorded in the literature demand notice here.

F. tusca and *F. valdarnensis* are described by Dequal (1914, pp. 15, 17) as having setae which are sigmoid but those of a bundle are of unequal length, the inner ones being shorter. The description is very meager but it appears that even though they be sigmoid, they still resemble the typical *Fridericia* arrangement and development. Stephenson (1915, p. 47) describes the setae of *F. carmichaeli* as being of the "*Enchytraeus type*" but it may be that since in this species there are usually only two setae per bundle and since the outer setae of a *Fridericia* bundle are straight and approximately the same size, this statement could be true, although if more were present per bundle the inner ones might be shorter, smaller, and arranged in pairs. Friend (1912b, p. 24) states that the head pore in *F. anglica* occurs on the tip of the prostomium. In *Fridericia peruviana*, Friend (1911, pp. 734–736) described the oesophagus as passing abruptly into the intestine, but since the specimens on which the description was based

were immature, it seems best to attach no particular significance to this case. According to Southern (1909, p. 165) *F. magna* Friend has bright red blood. Friend (1899, p. 263) stated that in *F. magna* an ovisac is present, extending caudad to 16.

At present about 90 species are assigned to *Fridericia* and while it is very possible that some of them are not valid, it appears that this is the largest of all the enchytraeid genera.

DISTICHOPUS LEIDY

Setae in two bundles per somite, representing the ventral rows only; nearly straight; simple-pointed but blunt; very stout and swollen in middle; hooked at proximal end. Head pore at 0/1. Oesophagus merging gradually into intestine. Peptonephridia present. Origin of dorsal blood-vessel postclitellar; small cardiac body present. Blood colorless. Nephridia with small anteseptal part. Spermiducal funnel cylindrical; sperm duct short, confined to 12. Penial bulb of lumbricillid type. Spermathecae not described.

Discussion

The genus *Distichopus* is known only from a single set of specimens collected in Delaware and Pennsylvania by Leidy (1882, pp. 146–147). Some of these specimens were later studied by Moore (1895, pp. 754–756) who extended the account, although even yet too little is known concerning this unusual form. Certain important structural features, such as the spermathecae, are yet undescribed and thus the relationships of this genus are difficult to determine. Moore holds that it is a close ally of *Fridericia*. The single known species bears the name *silvestris*.

ACHAETA VEJDOVSKY

Setae entirely absent; dorsal and ventral rows preserved in some species only as pear-shaped gland cells in body-wall, gland cells also absent in other species. Head pore large; on tip of prostomium. Dorsal pores absent. Oesophagus merging gradually into intestine. Peptonephridia present or absent. Origin of dorsal blood-vessel anteclitellar. Blood colorless. Nephridia with moderate or large anteseptal part. Spermiducal funnel cylindrical; sperm duct short or long but confined to region of clitellum. Penial bulb present; struc-

ture practically unknown; probably of lumbricillid type. Spermathecae with or without connection with digestive tract; diverticula absent.

Discussion

Achaeta is a small genus to which is assigned, at the present time, eight species, none of which occur in the Western Hemisphere. Its most striking characteristic is the total absence of setae. In most of the species, specialized, pear-shaped seta-glands occur in the positions where setae would be expected, although three species, *vejdovskyi* Bretscher (1902, p. 27), *maorica* Benham (1904a, pp. 221–223) and *camerani* (Cognetti 1899, pp. 1–4) are described as being completely devoid of seta-glands. Peptonephridia have not been found in *minima* Southern (1907, p. 77) and *incisa* Friend (1914b, pp. 133–134) but occur in the other known species. The penial bulb is practically unknown for this group since in no case is it described in adequate detail. It was figured by Vejdovsky (1879, pl. I, fig. 11) for *eisenii* but even there it is difficult to determine its exact composition. It suggests the lumbricillid type of bulb. In *maorica* Benham (1904a, p. 222) the spermathecae are greatly elongated, extending to 9 or 10, thus recalling the greatly elongated spermathecae of some of American species of *Mesenchytraeus*.

ENCHYTRAEUS HENLE

Setae straight; those of a bundle equal; distal extremities simple-pointed; four bundles per somite, two lateral and two ventral. Head pore at 0/1. Dorsal pores absent; Oesophagus merging gradually into the intestine. Peptonephridia present or absent. Intestinal diverticula absent. Chylus cells absent. Origin of dorsal blood-vessel postclitellar or intraclitellar; cardiac body absent. Blood usually colorless. Nephridia with small anteseptal part; cell mass well developed. Ventral glands sometimes present. Testes not divided. Spermiducal funnel cylindrical; Sperm duct confined to 12, or quite long, extending caudad through several somites. Sperm sacs present or absent. Penial bulb of enchytraeid type. Spermathecae connecting with digestive tract; diverticula present or absent.

Discussion

While it has been necessary to modify the older definition of the genus, only a few points require mention here. *E. dubius* (Stephenson, 1911, p. 56) is unique in possessing testes which are divided very much as is the case in representatives of the genus *Lumbricillus*. However, Stephenson himself (1915, p. 43) indicates that there is some doubt as to the generic position of this species. The same writer (1915, pp. 43–44) gives a critical discussion of sperm sacs in the genus *Enchytraeus* but makes no attempt to draw a general conclusion. Since the matter of sperm sacs is still in doubt, it seems best, in this paper, to use the data directly from the original descriptions and consider the statements of absence of sperm sacs as valid until they are definitely shown to be in error. The writer (Welch, 1914, pp. 177–178) previously discussed the penial bulb as a generic character and pointed out that not all of the species included in *Enchytraeus* conform to the enchytraeid type of bulb. However, it appears at this time that in the cases in which the penial bulb has been adequately described the large majority have bulbs of the enchytraeid type as proposed by Eisen and may so be incorporated into a revised statement of the limits of the genus, at least until subsequent investigation yields more complete data.

Eisen (1905, p. 61), in his generic description, states that the intestine generally possesses chylus cells. However, no mention of these cells is made later in his descriptions of species.

A genus, *Litorea*, described by Čejka (1913) for the reception of a species which he called *krumbachi*, is certainly the same as *Enchytraeus* and is so treated in this paper.

About thirty-five species are considered as belonging to this genus at the present time.

MICHAELSENA UDE

Setae straight; distal extremity simple-pointed; one to two setae per bundle, often but one; four bundles per somite, two lateral and two ventral; present only on some of the somites (except in *M. mangeri* Mchlsn.). Head pore at 0/1. Dorsal pores absent. Oesophagus merging gradually into intestine. Peptonephridia present or absent. Intestinal diverticula absent. Origin of dorsal blood-vessel postclitellar; cardiac body absent. Blood colorless. Nephridia with

small anteseptal part, consisting of nephrostome only. Ventral glands absent. Testes not divided. Spermiducal funnel cylindrical; sperm duct confined to 12, or long and extending caudad to 14. Penial bulb of the enchytraeid type. Spermathecae connecting with digestive tract; diverticula absent.

Discussion

Formerly the absence of setae from some or the majority of the somites was regarded as one of the chief distinguishing features of this genus but Michaelsen (1914, pp. 177–181) described a new species under the name *mangeri* in which setae are present in four bundles on all of the somites. This species may be regarded as a connecting form between *Michaelsena* and *Enchytraeus* and at the same time, according to Michaelsen, emphasizes the relation of *Michaelsena* to *Fridericia*.

Southern (1913, pp. 8–12) described under the name *Grania* what he regarded as a new genus, pointing out similarities with a form then known under the name of *Enchytraeus monochaetus* Mchlsn. The latter is now known to belong to *Michaelsena* and *Michaelsen* (1914, p. 181) seems to be right in placing *Grania* there also.

Eisen (1905, p. 73) incorporated in his definition of this genus the statement that there are "No penial bulbs," but this seemed to be based upon the condition which he found in a single specimen to which he gave the name *paucispina* and which did not permit a full descriptiption. However, the descriptions of species now assigned to *Michaelsena* indicate that it may be of the enchytraeid type. Eisen (1905, p. 11) placed the genus in his subfamily *Enchytraeinae*.

It seems to be becoming increasingly difficult to separate *Michaelsena* from *Enchytraeus* and it is possible that some future revision based upon more intensive study of all of the species involved may suggest the fusion of the two groups.

Eight species are assigned to this genus at present.

MESENCHYTRAEUS EISEN

Setae sigmoid; distal extremities simple-pointed; approximately equal in size in bundle; four bundles per somite, two lateral and two ventral. Head pore distinct; usually at or very near tip of prostomium. Dorsal pores absent. Oesophagus merging gradually into

intestine. Peptonephridia absent. Intestinal diverticula absent. Chylus cells absent. Origin of dorsal blood-vessel postclitellar; cardiac body present. Blood either colorless or red. Nephridia with small anteseptal portion, consisting merely of nephrostome; postseptal part large, irregularly pluri-lobed, and with cell mass between folds of closely wound nephridial canal greatly reduced. Ventral glands absent. Testes compact; undivided. Spermiducal funnel usually cylindrical; sperm duct short and confined to 12, or very long extending caudad for many somites. Sperm sacs and an ovisac often present. Penial bulb of mesenchytraeid type. Spermathecae confined to 5, or elongated and extending caudad for varying distances, sometimes to clitellum; diverticula present or absent; communication with digestive tract present or absent.

Discussion

Eisen (1905, p. 14) stated that a "single median ovisac" and "one pair of sperm-sacs generally of large size" are present in *Mesenchytraeus*. However, *Mes. altus* Welch (1917, p. 71), which unquestionably belongs to this genus, has a *pair* of ovisacs and it seems possible that other cases of that sort will appear.

Several special cases which depart somewhat from the definiton as proposed require mention here. In *eastwoodi* Eisen (1905, p. 50) the head pore occurs on the upper side of the prostomium near 0/1. *Mes. mencli* Vejd. (1905, p. 5) is described as having "Herz im 12," apparently referring to an intraclitellar origin of the dorsal blood-vessel. A similar origin seems true of *celticus* Southern (1909, p. 155), although the statement is not made positively. Bretscher (1902, p. 16) claimed that specimens of *setosus* Mchlsn. (=*megachaetus* Bret.) show the dorsal blood-vessel arising in 11, 13, or 16 — which seems an unusual variation. *Mes. grandis* Eisen (1905, p. 44) is described as having nephridia with broad anteseptal parts. In *orcae*, *mirabilis*, and *kincaidi* (Eisen, 1905, pp. 40–41), the testes are recorded as composed of "lobes" but it is not clear whether they are deeply divided as in the case of *Lumbricillus* or are merely lobulate at the free extremity. Bretscher (1901, p. 212) states that in *alpinus* the spermiducal funnel is in 8, but nothing is given concerning the sperm duct. In *nanus* (Eisen, 1905, pp. 51–52), the penial bulb is described

as absent, but there seems to be some possibility that immature specimens were studied.

This genus contains at the present time about 50 species.

HYDRENCHYTRAEUS BRETSCHER

Setae sigmoid; distal extremities simple-pointed; four bundles per somite, two lateral and two ventral. Dorsal pores absent. Oesophagus merging gradually into intestine. Peptonephridia present. Origin of dorsal blood-vessel postclitellar. Blood yellow or red. Nephridia with large or small anteseptal part. Spermiducal funnel cylindrical. Spermathecae without diverticula.

Discussion

This genus was established by Bretscher (1901, pp. 208–209) for two incompletely described species, *stebleri* and *nematoides*, found in Switzerland. Its status is somewhat uncertain owing to the fact that information on the head pore, relation of oesophagus to intestine, chylus cells, cardiac body, ventral glands, testes, sperm sacs, ovisacs, penial bulb, and relation of spermathecae to the digestive tract is entirely lacking. Likewise certain other features are incompletely described. Since the original description is the only record, nothing further can be done with these forms until specimens are again found and studied critically. The fragmentary information which is available seems to indicate that it is a valid genus.

STERCUTUS MICHAELSEN

Setae sigmoid; distal extremity simple-pointed; four bundles per somite, two lateral and two ventral. Head pore absent (apparently) or very small. Dorsal pores absent. Oesophagus merging gradually into intestine. Peptonephridia absent. Intestinal diverticula absent. Chylus cells absent. Origin of dorsal blood-vessel anteclitellar; cardiac body present. Blood colorless. Nephridia with small anteseptal part consisting of but little more than a mere nephrostome; postseptal part large; nephridial canal loosely wound and surrounded by considerable cell mass. Testes small; undivided. Spermiducal funnel short and distinctly funnel-shaped. Penial bulb unknown. Spermathecae not connected with digestive tract; diverticula absent.

Discussion

The genus *Stercutus* was established by Michaelsen (1888) to receive a species, *niveus*, which was found inhabiting fish excrement in Germany. No other representatives of this genus have ever been described. Lack of information as to the character of the penial bulb, the sperm duct, and the presence or absence of ventral glands, ovisacs and sperm sacs gives some difficulty in determining the affinities of this genus.

CHIRODRILUS VERRILL

Setae in six bundles per somite, two sub-dorsal, two lateral, and two ventral; those in ventral and lateral bundles distinctly sigmoid, those in sub-dorsal less curved; distal extremities simple-pointed. Blood colorless.

Discussion

The description of this interesting genus is extremely meager and based entirely upon the original record by Smith and Verrill (1871, pp. 450–451) in which but few of the important details are described. Two species are assigned to this genus, *larviformis* and *abyssorum*, both collected in Lake Superior. Both are apparently deep water forms, *larviformis* being dredged from depths of 17 and 59 fathoms, and *abyssorum* from 47 and 159 fathoms. There is some question as to the position of this genus, certain previous writers having regarded it as a tubificid. Beddard (1895, p. 314) and Michaelsen (1900, p. 88) have classed it among the Enchytraeidae, although the latter (1903, p. 50) later placed it among the Tubificidae. Eisen (1905, p. 13) retains it in the Enchytraeidae. It is unlikely that the matter can receive any positive decision until material is again secured and carefully studied. Since some of the characters as described are apparently enchytraeid in nature, it is included in this review, with the realization, however, that future investigation may show it to have other affinities. If it be an enchytraeid, it is unique for the entire family in possessing six sets of setae per somite. Eisen (1905, p. 13) places it under the Lumbricillinae, but states (p. 6) that it is "appended for convenience sake" and points out correctly that nothing is known concerning the penial bulb and other internal structures.

GENERA DUBIA

Under the name *Chamaedrilus*, Friend (1913a, pp. 260–263) described a new genus from material collected in England. Considering the generic characters as recognized at present for the family Enchytraeidae, a careful comparison has made it impossible for the writer to distinguish *Chamaedrilus* from *Marionina* and in this paper they are regarded as the same.

Bretscher (1905) described what he regarded as a new genus of Enchytraeidae under the name of *Euenchytraeus* but unfortunately the record was made on sexually immature material and as a consequence nothing could be determined as to the nature of the reproductive organs. Since no further record of this postulated genus occurs in the literature and since the original description is unusable as it stands, it is omitted from consideration in this paper.

KEY FOR THE IDENTIFICATION OF THE GENERA OF ENCHYTRAEIDAE

1 (2) Setae entirely absent; represented in most species only by four longitudinal rows of pear-shaped glands in body-wall ..... *Achaeta*

2 (1) Setae present 3

3 (4) Setae arranged in two bundles per somite ..... *Distichopus*

4 (3) Setae arranged in more than two bundles per somite 5

5 (6) Setae arranged in six bundles per somite, two subdorsal, two lateral, and two ventral ..... *Chirodrilus*

6 (5) Setae arranged in four bundles per somite, two lateral and two ventral 7

7 (8) Setae cleft at distal extremities; spermiducal funnel wide, open, shallow, and extremely short; postseptal part of nephridia composed of a few coherent folds not intimately fused, forming very loose organ ..... *Propappus*

8 (7) Setae simple-pointed at distal extremities; spermiducal funnel cylindrical or trumpet-shaped; postseptal part of nephridia compact 9

9 (10) Setae straight, arranged in pairs, inner pairs of bundle successively smaller than outer; dorsal pores present; chylus cells present in walls of intestine ..... *Fridericia*

10 (9) Setae straight, sigmoid, or in pairs in bundle with smaller ones within; dorsal pores absent; chylus cells absent from walls of intestine.....11
11 (14) Oesophagus expanding abruptly into intestine.....12
12 (13) Dorsal blood-vessel arising from anterior end of single, dorsal intestinal diverticulum.....*Buchholzia*
13 (12) Dorsal blood-vessel arising directly from perivisceral blood-sinus..... *Henlea**
14 (11) Oesophagus merging gradually into intestine.....15
15 (16) Setae usually absent from several somites (except in *Michaelsena mangeri* Mchlsn.); usually one setae per bundle, never more than two.....*Michaelsena*
16 (15) Setae regularly present on all somites except first and last and possibly the clitellar.....17
17 (20) Intestinal diverticula present.....18
18 (19) Setae sigmoid; four distinct intestinal diverticula; origin of dorsal blood-vessel intraclitellar.....*Bryodrilus*
19 (18) Setae straight; one intestinal diverticulum completely surrounding digestive tract; origin of dorsal blood-vessel anteclitellar..... *Hepatogaster*
20 (17) Intestinal diverticula absent.....21
21 (22) Setae straight; those of a bundle equal.....*Enchytraeus*
22 (21) Setae sigmoid.....23
23 (24) Peptonephridia present.....*Hydrenchytraeus*
24 (23) Peptonephridia absent.....25
25 (26) Origin of dorsal blood-vessel anteclitellar; spermiducal funnel short and trumpet-shaped.....*Stercutus*
26 (25) Origin of dorsal blood-vessel postclitellar; spermiducal funnel cylindrical.....27
27 (28) Testes divided.....*Lumbricillus*
28 (27) Testes solid, not divided.....29
29 (30) Cardiac body absent; penial bulb of lumbricillid type; nephridial duct loosely coiled and cell mass of postseptal part well developed; spermathecae never extending through several somites.....*Marionina*

* A few species, e.g., *hillmani* Fr., *insulae* Fr., *marina* Fr., *alba* Fr., and three or four uncertain forms, have been assigned to *Henlea*, although they are described as having the oesophagus pass gradually into the intestine.

30 (29) Cardiac body present; penial bulb of mesenchytraeid type; nephridial duct closely wound and cell mass reduced to minimum..... *Mesenchytraeus*

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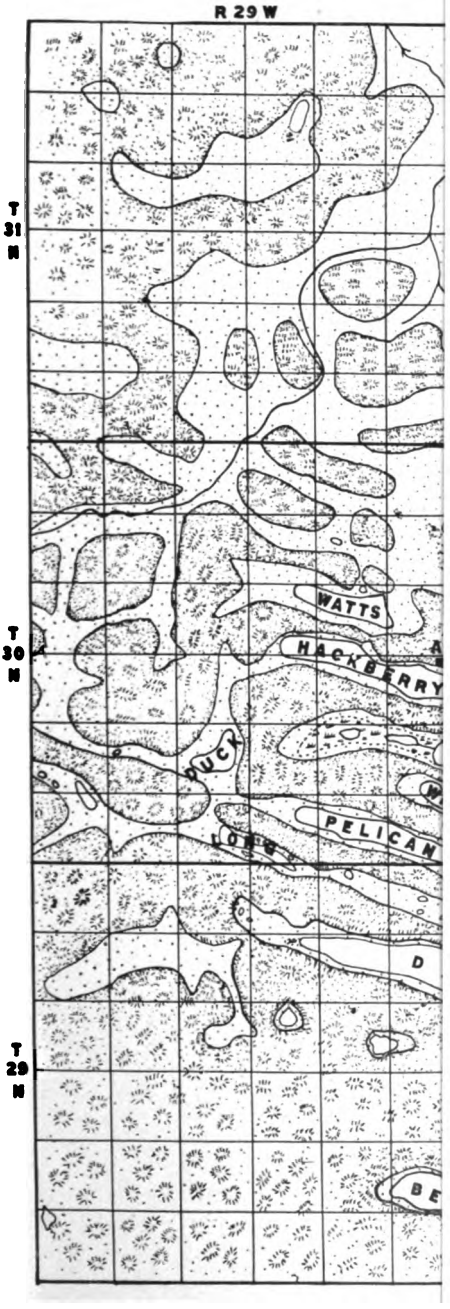

PLATE III

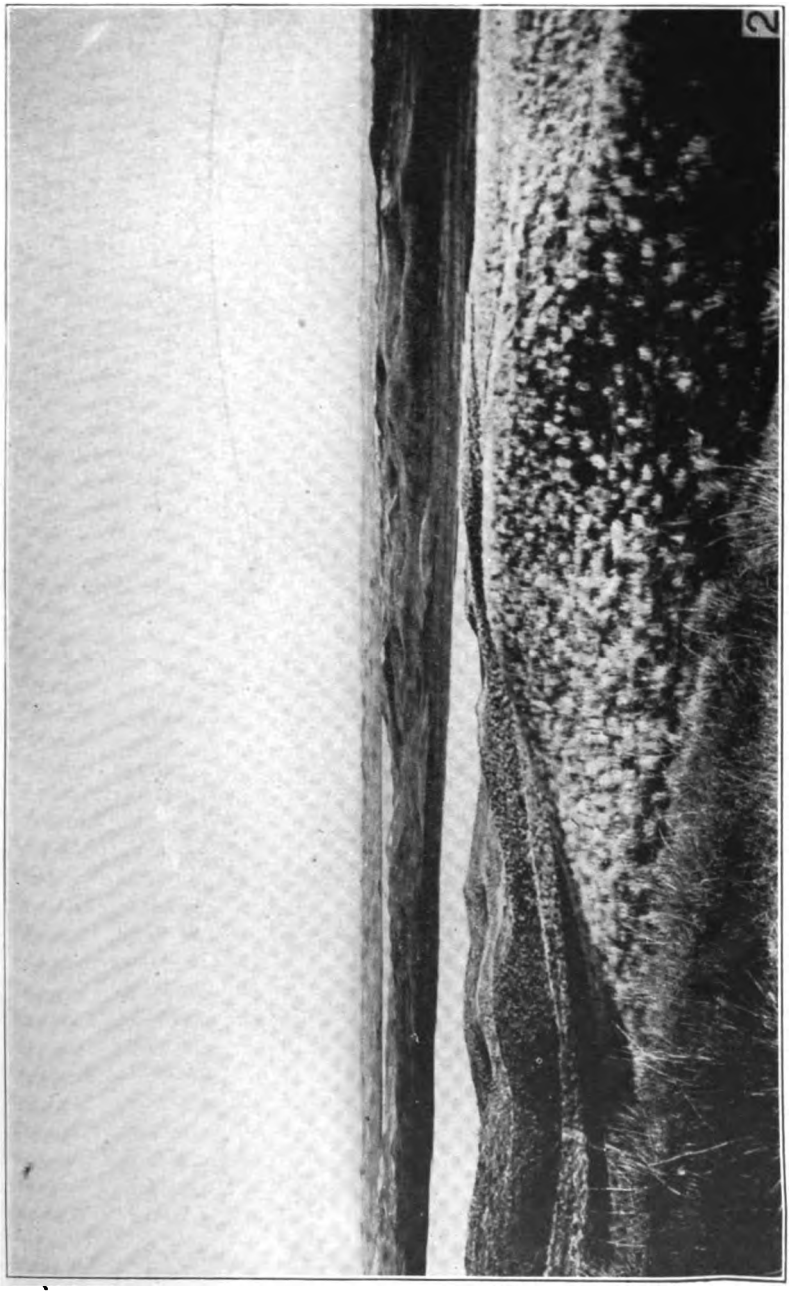

PLATE IV **ANDERSEN AND WALKER**

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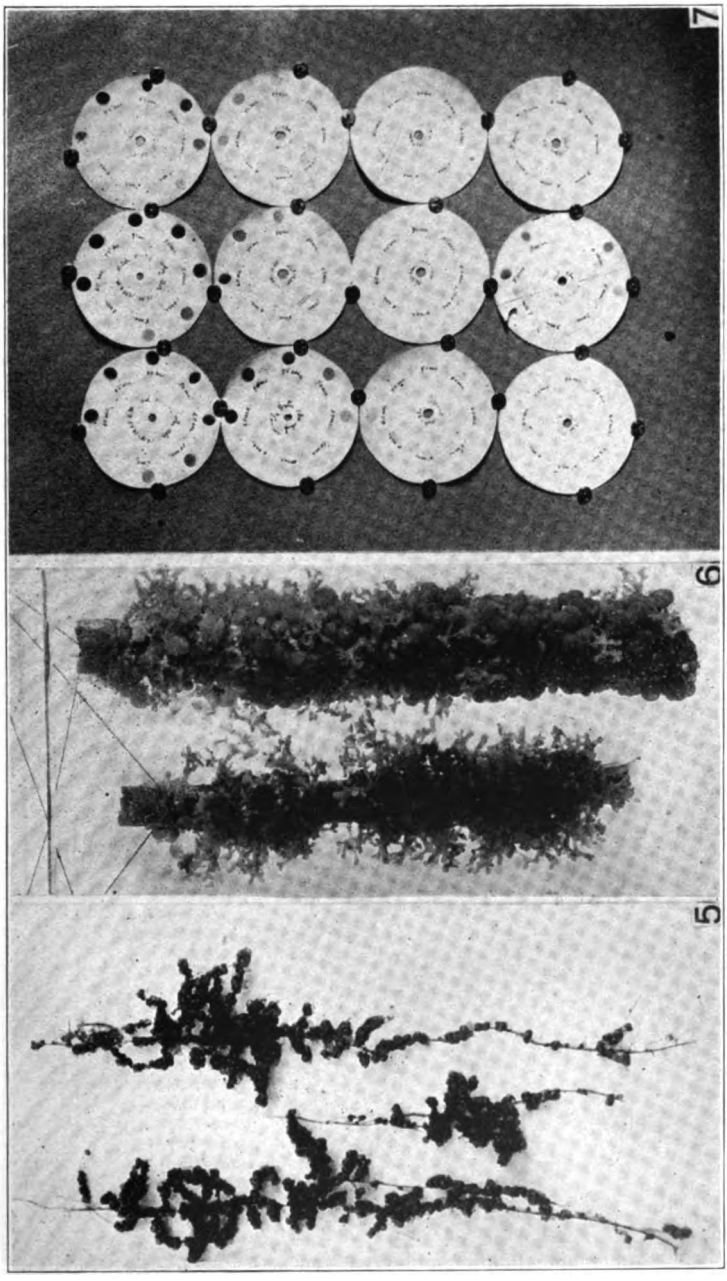

PLATE VI **ANDERSEN AND WALKER**

AN ECOLOGICAL STUDY OF THE ALGAE OF SOME SANDHILL LAKES

By Emma N. Andersen and Elda R. Walker

INTRODUCTION

Much of the western part of Nebraska consists of rolling sandhills covered with bunchgrasses, yuccas, cacti, and other dry-land plants. Cherry county which is situated in the north central part of the sandhills has in its valleys many bodies of water. In one area of 250 square miles there are about 75 lakes. This region is twenty-five miles southwest from the village of Woodlake and about the same distance south of Valentine. These lakes vary greatly in size. Some are ponds a few hundred feet in diameter while the largest of the group is about four and a half miles long and three-fourths of a mile wide. All are comparatively shallow bodies of water, varying in depth from a couple of feet to a maximum of fifteen feet. The accompanying map[1] (Fig. 1) shows the relative size and arrangement of the lakes of this region.

Surrounding the lakes are low meadows covered with grasses and prairie flowers.[2] These meadows extend back from the lakes anywhere from a rod to, in some cases, a mile or more. Surrounding the grasslands rise the "sandhills"—dunes of yellowish sand extending to the next lake with its surrounding grasslands (Fig. 2). The few native trees are stunted and produce no effect on the landscape.

The climate is that typical of the central plains, dry, windy, hot in summer, and cold in winter. In the summer of 1912, the average maximum daily temperature from June 24th to August 3rd was 91.2°, while the average minimum temperature was 59.8°. During the same period the wind velocity reached a maximum of 21.9 miles per hour, the average for a twelve hour period, while the lowest average for a similar period was 1.3 miles per hour.

[1] Furnished by Dr. G. E. Condra of the University of Nebraska.

[2] The vegetation of this region is well discussed by Pool (36).

The high daily temperature warms the water of the shallow lakes and the prevailing high winds stir it, frequently mixing the warm surface layers with those below causing thorough aëration. The climatic conditions together with the abundance of water plants, such as *Chara*, *Myriophyllum*, *Potomogeton*, *Scirpus*, *Nymphaea*, and *Zizania*, which give anchorage for attached forms, make an ideal habitat for algae.

Although complete analyses of the water of the lakes are not available, some idea of their alkalinity may be obtained from analyses made by the department of chemistry of the University of Nebraska of samples of water taken by Dr. R. H. Wolcott. These were made in 1911 and showed the following parts per million of alkali:

Watts Lake, 111
Dewey Lake, 160
Hackberry Lake (no analysis made)
Big Alkali Lake, 622
Clear Lake, 1,129

As the analyses show some of the lakes are adapted to the algae of fresher waters, while others are so alkaline that very few forms can inhabit them. There is evidence that all of the series of lakes belonged formerly to one general system. The larger lakes have well formed shore lines except at their northwest ends where many of them are swampy. This gives farther variations in the habitat for algae.

Surrounded by a large semiarid region, these lakes with their large beds of wild rice and other seed producing plants prove a most tempting resting and feeding place for migratory birds. They flock here in large numbers and, no doubt, bring on their mud-laden feet spores of algae from ponds both north and south—the only explanation for some of the species present.

Earlier students working with the higher plants of the sandhills reported a rich algal flora. This led the writers to spend the summer of 1912 in studying the algae of the region and the conditions under which they live. At first it was hoped to cover the entire group of lakes, but after a few preliminary trips through the region, the work was limited to a few localities so situated that they could be frequently visited. These were chosen to represent so far as possible the different types of habitat found in the region.

From the various localities, specimens were collected by hand either from a boat or by wading. A Birge net was also used to secure free floating forms.

The identifications of species are based upon Tilden's Myxophyceae, Collins' Green Algae of North America, and West's British Desmidiaceae. In groups such as the Oedogoniaceae, Characeae, and Helminthocladiaceae covered by special publications, the identifications were made with the works cited. Constant reference was made to the other systematic works listed at the end of this paper.

The nomenclature of DeToni is followed except for the Desmids where that of West is used. In the case of a few species not given in the above general works, the terminology of the author describing the form is used.

All diatoms were identified by Dr. C. J. Elmore. The one Volvox found was identified by Dr. J. H. Powers. Acknowledgments are also due Mr. F. H. Shoemaker for the photographs from which figures 2, 10, 11, and 12 were taken; to the Nebraska Conservation and Soil Survey for help in prosecuting the work; to Prof. B. E. Moore for suggestions as to physical problems; and to Prof. T. J. Fitzpatrick for careful reading of manuscript and proof.

HACKBERRY LAKE

Hackberry lake (Fig. 1, 3) was chosen for a study of water conditions, because it was representative and conveniently located. It is a lake two and a half miles long and one-half mile wide. In depth it varied during the summer of 1912 from three to seven feet. The maximum depth, however, was found only in a few places. Usually it did not exceed four feet.

The shore is sandy except at the northwest end where it is freshly formed by the filling in of decomposing vegetable matter. This end is swampy and passes gradually into dense beds of *Zizania*, *Scirpus*, *Myriophyllum*, *Potomogeton*, and similar water plants. Here the water is so filled with vegetation that it is almost impenetrable with a boat or otherwise.

The southeast half of the lake was more open. Dense beds of water plants were scattered through it but between them were areas of open water, (Fig. 4). Here the algae were most abundant. It was in this region that records of water conditions were made.

Algae were abundant all over the lake but more so in this part where every rush stem, every lily pad, in fact, every submerged plant was loaded with them (Figs. 5, 6). The number of species was not large, as the list which follows shows, but the number of individuals was very great. For example, a count was made of the thalli of *Chaetophora elegans*. On one old Scirpus stem there were 592 thalli. In an area one meter square, there were 103 stems loaded in a similar way, making over sixty thousand thalli of *Chaetophora elegans* in a square meter. *Chaetophora cornu-damae*, *Nostoc glomeratum*, *Gongrosira debaryana*, and *Rivularia natans* were equally abundant, while other species were only slightly less so.

Climatic Conditions

The accompanying graphs (Figs. 15, 16, 17) are intended to illustrate weather conditions surrounding the lake. The station, (C on Fig. 1) represented in Fig. 8, was in a blowout about ¼ mile from the lake. In the graph (Fig. 15) line A shows the daily variation in temperature of this station as recorded by a Fries self-registering thermograph. Line D shows the temperature on the north side of a small house, a few rods from the lake (A on Fig. 1) as registered by a maximum and minimum thermometer. Line C shows the temperature of the surface sand as recorded by a Fries self-registering soil thermograph whose bulb was barely covered with sand. B gives the temperature of the sand eight inches below the surface as recorded by a similar instrument. The wind record was made by a standard anemometer (Julien P. Fries), so only the averages of wind velocity for twelve hour periods are available, but it gives some estimate as to wind in the region. The high air temperature and its effect on the surface temperature of the light sand when compared with the temperature of the sand eight inches below the surface shows that comparatively little heat penetrates deeply into the soil—a fact that may have some bearing on the temperature of the water of the lakes in some instances. Fig. 16 is a similar record for a much cooler week taken in the grass of the meadow (Fig. 9) on the lake shore (B on Fig. 1).

WATER CONDITIONS

Temperature and Wind

For a month, July 8 to August 6, the same instruments were stationed in a boat on this lake to determine, so far as possible, the temperature conditions under which the algae were growing. During this time the anemometer was placed on a post about six feet above the surface of the water and a hundred feet from the water's edge to determine the velocity of the wind passing over the water. The thermographs in the boat recorded the temperature (*A*) of the air in the boat, the temperature (*C*) of the surface water, and the temperature (*B*) of the water at the bottom of the lake. Due to drifting of the boat to a slightly different position, the depth at which the temperature was taken varied from three feet the first week to four and one-half the second and fourth weeks and five feet the third week. The temperatures were not taken in deeper parts of the lake because there the algal growth was slight. As can be seen from the accompanying graph (Fig. 17) the temperatures of the surface and bottom water approach each other very closely except on days of low wind, when the temperature of the surface water varies considerably from that at the bottom. This effect of air temperature and wind was especially evident in the fourth week when great variations in air temperature occurred.

It will be noticed that during a large part of this month, the water temperatures were between 70° and 80° F., and that the temperature of the water at all levels was remarkably uniform. It is evident from the divergences of the temperature of surface and bottom water during each period of lower wind that the uniformity of the water temperature is due largely to stirring by wind. Also that a constant stirring would produce good aëration in all parts of the water is an inevitable conclusion.

Hackberry lake is one of the less alkaline of the lakes in this group. It is, however, more alkaline than some of its neighbors as was noted before.

Light

A modification of the common solio paper photometer (text Fig. 1) was devised to study the light conditions under which the algae were

growing.[1] This consisted of a water tight circular drum (A) at the end of a metal tube (D). Inside of this drum was a disk (G) which revolved by means of a rod (E) passing through the tube and connected with a lever at the top. This disk (G) carried the paper (F) on its under surface. It was perforated by eight holes (B) of the same size as a clear glass window (C) in the drum. By revolving the inner disk, the areas of solio paper under the perforation of the disk could be exposed to the light through the window (C). The tube and

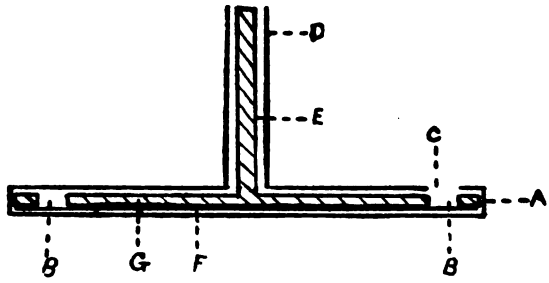

Fig. 1

rod inside of it were made in sections so they could be extended to the necessary length. The tube was marked in decimeters on the outside so the depth could be ascertained at all times. With this instrument, light readings were taken from a boat (Fig. 4) at various depths. Care was taken to have the window in the drum exposed to direct light at all times. In some instances, the period of exposure was uniform and the depth was varied; in other cases both depth and period of exposure were varied and in still others one depth was maintained during the series of readings and the period of exposure was varied.

Records taken by the last method are shown in (Fig. 7). The top row shows exposures made in the air beginning at the following hours, reading from left to right: 10:18 a.m., 10:30 a.m., and 2:10 p.m. In each record the exposures were 60, 45, 40, 30, 20, 10, 5, and 2 seconds.

Below are nine water records taken the same day and at intervals immediately following or preceding the air exposures. These are

[1] This instrument was made by the Spencer Lens Company.

arranged in order by depths, the first being one decimeter below the surface and each succeeding one a decimeter deeper, making the last nine decimeters deep. In all the periods of exposure were the same as the air records, except the last two. Here no records could be secured at the above exposure periods so the time was increased in record 8 to 2, 3, 4, 5, 6, 7, and 8 minutes and in record 9 to 1, 2, 3, 4, 5, 6, 7, and 8 minutes. All water readings were compared with exposures made in the air at the same hour. In no cases were the readings satisfactory but some facts of interest were recorded.

1. Waves on the surface water varied the record of light intensity greatly. On very windy days the intensity of light under the water was so variable that one exposure showed almost no coloration while the next at the same depth and period of exposure was deeply colored. The only explanation evident was that the change in angle of surface layer deflects the light almost entirely at times and not at other times. On account of this variability readings on windy days were early discontinued.

2. Before 9 A.M. or after 3 P.M. it was almost impossible to get a usable tint on the solio paper, although to the eye it was entirely light to the bottom of the lake. Evidently the angle between the sun's rays and the surface of the water was such that most of the rays were deflected and the water light was simply reflected light and not of a kind or quantity to affect solio paper.

3. Exposures made at a given depth but with different periods of exposure gave very different records as to relative light intensity. The shorter the exposure, the greater reduction of light, according to the readings. When the exposure required more than three minutes the error was so great that the reading was useless for comparison with readings of shorter periods. Two series will illustrate the point. The exposures were consecutive in both cases.

Time	Relative intensity All at 4 dm. deep	Time	Relative intensity All at 8 dm. deep
3 min.....	.111	4 min.....	.0083
2 min.....	.125	5 min.....	.0166
1 min.....	.083	6 min.....	.0277
½ min.....	.05	7 min.....	.0357
		8 min.....	.0416

Such a series can only be explained by the well known fact that the reduction of silver in a photographic paper is not a uniform process. Since these results proved a variable period impracticable, exposures were made for a given period at various depths. These readings showed clearly a constant reduction of light as the depth was increased and agreed in general with the results obtained by Needham and Lloyd (33), by Birge (11), and by Oltmanns (34).

4. Exposures made for constant periods at various depths showed the light to be reduced by passing through the water. However, this gave no accurate measure of the light at given depths, because such exposures were compared with air tints of various periods of exposure. These we have just shown to be unreliable because of the inconstant reduction of the photographic film.

5. Solio paper is sensitive only to the blue-violet end of the spectrum. It is generally conceded that it is the red-orange end of the spectrum that is most largely used by plants in photosynthesis (Dangeard 22). It is, therefore, evident that tests made with solio paper are of little value in determining the light conditions under which algae grow. Such tests show in a most general way, as noted above, that the direct rays of light are largely deflected except during the middle of the day and that the light is reduced by passing through water. Both facts are well known to physicists. This means that water plants have a shorter day than air plants and that they grow in less intense light at all times. In other words, as Oltmanns (34) states, they are all shade plants.

6. Light readings taken in October show less light penetrating the water than was found in July and August. The following table shows this as well as could be determined from solio paper records. Because of the reasons already given these records can only be regarded as a crude estimate. The figures given are compiled from a large number of readings so that the general diminution of blue light per decimeter of depth and the relative intensity between the light of midsummer and that of October are approximately correct.

Dm.	August	October
1	.5	.2
2	.25	.1
3	.173	.066

Dm.	August	October
4	.083	.05
5	.05	.033
6	.033	.016

No doubt the reduction of light in October is due to the rays of the sun striking the water more obliquely. Hence water plants have not only a shorter day but also a shorter growing season than land plants.

It is evident that the main advantage in the present attempt to measure light with reference to that used by plants under water is to prove the absolute uselessness of the solio paper method.

Later the same photometer was modified slightly so that plates sensitive to all kinds of light could be used in it (Figs. 10, 11, 12). A color screen was added and in that way an attempt was made to measure the red-orange light penetrating the water. Fig. 10 shows the upper side of the drum which holds the photographic plate and the detachable color screen over the exposure window. This is shown again in Fig. 12 (below) with the tube removed. At the top of Fig. 12 is shown the under side of the upper half of the drum. The inner disk shows two clips for holding the plate in place and eight perforations through which areas of the plate may be exposed to the light of the window. This disk is revolved, exposing alternately a perforation and a solid area, by a rod passing through the tube and connected with the lever at the top (Fig. 11). This apparatus for making exposures, while entirely rapid enough for solio paper, proved altogether too slow for the highly sensitive plates. The only results obtained indicated that red light penetrating many decimeters of water was too strong for more than instantaneous exposures on these plates. It is believed that a similar apparatus fitted with a shutter that would make possible exposures of a fraction of a second would come much nearer to giving an estimate of light available for the use of algae. Even here the reduction of the photographic film would not be uniform and some error would remain. Oltmanns (34) states that physicists have shown that the light from each part of the spectrum is deflected and absorbed differently by pure water. The water inhabited by algae is always modified in color and transparency both by its chemical content and by the presence of floating organisms giving turbidity to the water. An instrument such as the above would give an idea as to what effect such water conditions have upon

the quality of light used by algae even tho it did not give accurate information as to the quantity of light available for them.

Murray and Hjort (32) investigating light conditions in the ocean, by means of the Helland-Hansen Photometer and pan-chromatic photographic plates found that red light penetrated the water less than did blue and indigo. This agrees with the results cited by Oltmanns (34) for pure water. This suggests, in some cases at least, that actual light available for submerged plants is far below the amount indicated by the solio paper photometer.

Distribution

As is readily seen from the physical factors noted above, this lake is characterized by fairly uniform temperature, aëration, and alkalinity. There remain but two factors that can influence the distribution of algae in this lake—light and mechanical support. The distribution may be entirely explained by these factors.

Attached forms as *Rivularia pisum*, *Nostoc glomeratum*, etc., grow at a depth of about 3–4 dm. below the surface of the water. When they are near the margin of the lake they grow on small submerged sedges, chara, etc. In deeper water they grow on Potomogeton, Myriophyllum, and such taller plants, but at remarkably uniform distances below the surface. On the other hand, *Nostoc pruniforme*, lying free at the bottom of the lake, was only found near the margin where the water was two or at most three decimeters deep. At first such forms led to the opinion that there was a zonal arrangement about the shore such as is suggested by Comère (17) but the theory did not stand the test. The distribution was entirely a vertical one caused undoubtedly by the light intensity. There were several conspicuous examples which demonstrate this. *Chaetophora elegans* extended on Scirpus stems, almost its only support, over a distance of two decimeters but the growth was constantly most abundant in the upper region, within ½–1 dm. of the surface of the water. *Chaetophora cornu-damae* had almost the same distribution while *Nostoc glomeratum* made its best growth at a depth of 2–4 dm. or even deeper. Evidently it was crowded downward by the Chaetophora with which it grew. Early in the season the Nostoc and Chaetophora grew together. Later the Chaetophora occupied the upper zone while Nostoc grew in equal abundance lower down.

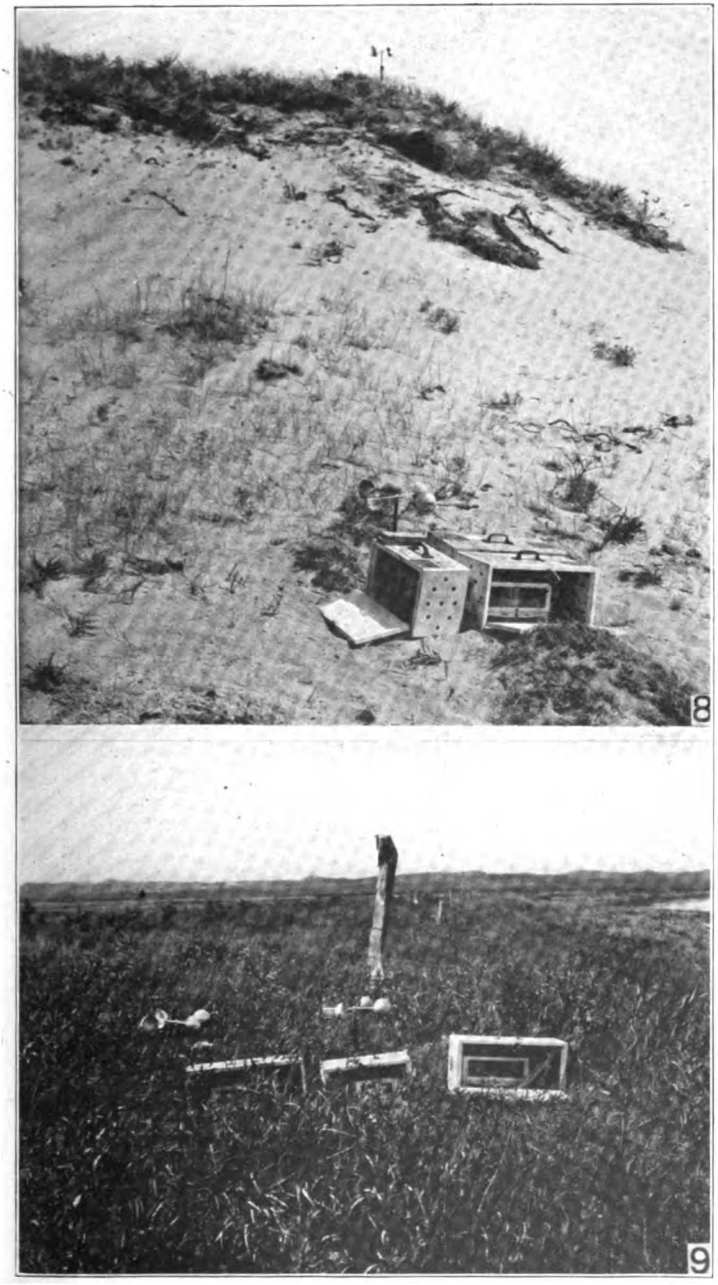

PLATE VII **ANDERSEN AND WALKER**

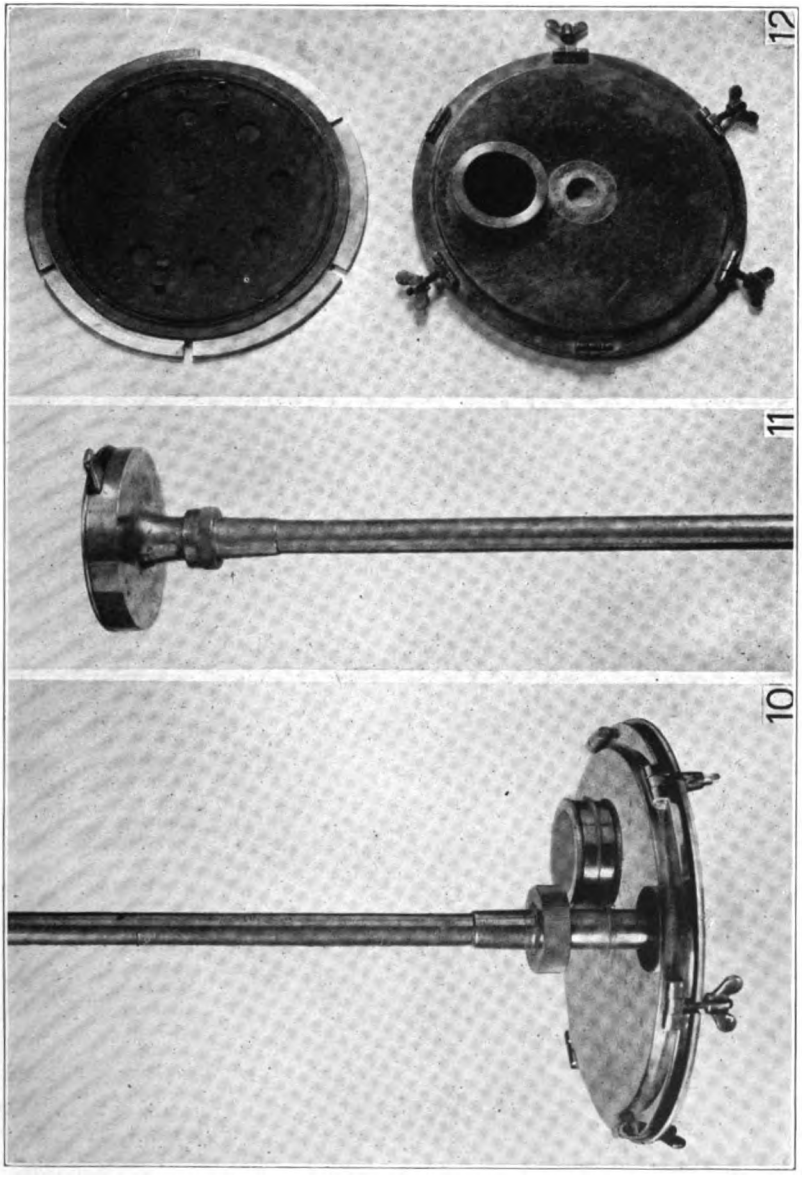

PLATE VIII **ANDERSEN AND WALKER**

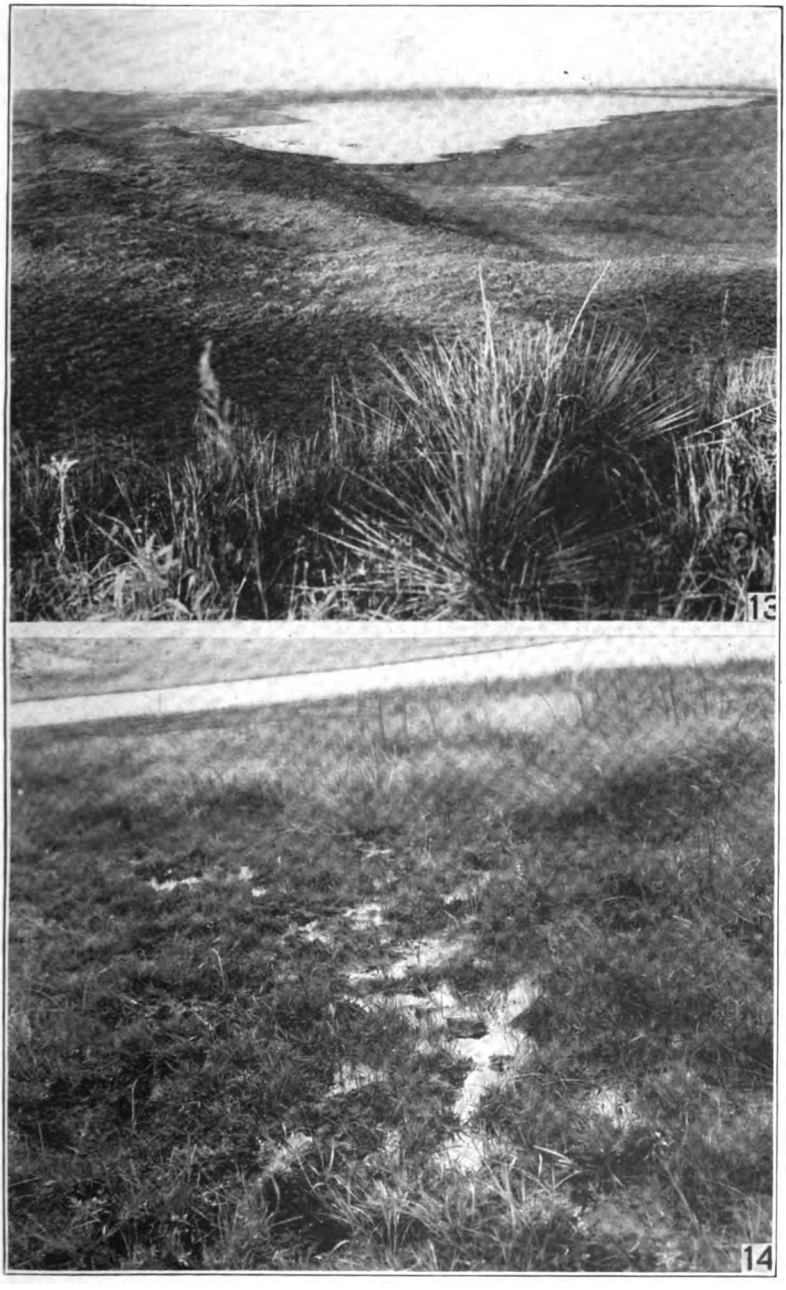

PLATE IX **ANDERSEN AND WALKER**

The following forms were found floating or attached almost exclusively in the upper two decimeters of water or in water not more than 2 dm. deep.

Clathrocystis aeruginosa	Microspora amoena
Coelosphaerium Kuetzingianum	Chaetophora cornu-damae
Nostoc muscorum	Chaetophora elegans
Nostoc humifusum	Bulbochaete sp.
Nostoc minutum	Oedogonium fragile
Nostoc zetterstedtii	Coleochaete orbicularis
Phormidium tenue	Gongrosira debaryana
Chara contraria	

Mixed with these were most of the unicellular forms of the yellow-green algae and most of the desmids listed for this lake.

Such forms as the following predominated in the deeper water, 2–4 dm. from the surface: *Rivularia natans*, *Rivularia pisum*, *Nostoc glomeratum*, and *Nostoc austinii*. With these were associated *Merismopedium aerugineum*, *Oscillatoria subtilissima*, *Dictyosphaerium pulchellum*, *Gloeocystis gigas*, *Scenedesmus quadricauda*, and *S. bijugatus*. The distribution of unicellular free floating forms, however, was hard to determine because of the constant stirring of the water. It will be noted that few yellow-green algae are characteristically found in the lower zone.

Below a depth of 4 dm. many algae were found but not in any charactertistic formation. Evidently they were there because crowded out elsewhere and they were not growing as well as the same species at higher levels.

As to grouping of the algae in these zones it was found that in any given location one or two species were dominant and others grew rather indiscriminately among them. The dominant species was apparently determined by the support on which it grew. In a bed of Scirpus, one species of *Chaetophora* and *Nostoc glomeratum* were almost universally the dominating forms, the Chaetophora dominating the upper and Nostoc the lower zone. On submerged mosses, Potomogeton, etc., *Gongrosira debaryana*, *Nostoc glomeratum*, or *Rivularia pisum* dominated, while at the margin of the lake a free floating form as *Nostoc pruniforme* or *Rivularia natans* might dominate. *Clathrocystis aeruginosa* or *Anabaena flos-aquae* usually

dominated the groups floating at the surface of the water, tho the constant winds kept these forms fairly well mixed.

Seasonal Distribution

Careful records of dates of collection were made to determine, so far as possible, the grouping of algae by seasons. As the study extended only from the middle of June until the middle of October, the observations included only summer and fall forms. Only the most general results were obtained. Of the species found in Hackberry lake the members of the Oscillatoriaceae were found only in the early summer, Merismopedium and Coelosphaerium only in July. Other members of the Myxophyceae were found in early stages in the first part of the season but reached full maturity and maximum abundance in October.

Most of the green algae were found throughout the period observed but they reached their maximum in midsummer except in a few cases. The species of Gloeocystis were found early in the summer. *Bulbochaete* was found late in the season (August–October) and the specimens were not fruiting. Nearly all the desmids were found during July. Only a few were present earlier or later in this lake, tho in places near by they occurred at other times in abundance.

The following table shows the forms found in the early, middle, and later summer and suggests something of the seasonal occurrence of the forms.

June 27–July 10	July 10–July 25	July 25–August
Nostoc humifusum	Nostoc zetterstedtii	Nostoc muscorum
Nostoc glomeratum	Nostoc pruniforme	Nostoc minutum
Nostoc austinii	Nostoc humifusum	Nostoc caeruleum
	Nostoc glomeratum	Nostoc pruniforme
		Nostoc glomeratum
Anabaena flos-aquae	Anabaena flos-aquae	Anabaena flos-aquae
Oscillatoria subtilissima		
Phormidium tenue		
Rivularia pisum	Rivularia pisum	Rivularia pisum
Rivularia natans (young)	Rivularia natans	Rivularia natans
	Merismopedium aerugineum	
	Coelosphaerium kuetzingianum	

June 27–July 10	July 10–July 25	July 25–August
Clathrocystis aeruginosa	Clathrocystis aeruginosa	Clathrocystis aeruginosa
	Staurastrum gracile	
	Spirotaenia obscura	
	Netrium digitus	
	Euastrum oblongum	
Cosmarium vexatum	Cosmarium subcrenatum	
Cosmarium obtusatum	Cosmarium retusiforme	
Cosmarium kjellmani grande		
	Cosmarium obtusatum	
	Cosmarium laeve	
	Cosmarium granatum	
	Cosmarium formosulum nathorstii	
	Cosmarium elfingii	
Closterium lanceolatum	Closterium pritchardianum	Closterium moniliferum
	Closterium leibleinii	
Chara contraria	Chara contraria	Chara contraria
Coleochaete orbicularis	Oedogonium fragile	Bulbochaete sp.
	Gongrosira debaryana	
Chaetophora elegans	Chaetophora elegans	Chaetophora elegans
Chaetophora cornu-damae	Chaetophora cornu-damae	Chaetophora cornu-damae
Gloeocystis vesiculosa	Microspora amoena	
Gloeocystis gigas		
Scenedesmus bijugatus	Scenedesmus quadricauda	
	Scenedesmus obliquus	
	Dictyosphaerium pulchellum	
	Pediastrium tetras	
	Pediastrum boryanum	
	Tetraedron trigonum	

A very interesting group of algae was found in a small ditch carrying water from a spring into the northwest end of Hackberry lake. *Closterium lunula* was so dominant here that at first it seemed to be the only species present. Closer examination, however, showed the following species associated with it.

Eremosphaera viridis	Cosmarium umbilicatum
Cylindrospermum majus	Euastrum verrucosum
Cosmarium angulosum concinnum	Pleurotaenium trabecula
Cosmarium circulare	Staurastrum meriani
Cosmarium pachydermum	Staurastrum punctulatum

Phormidium tenue, *P. valderianum*, and *Spirulina major* were found on the lake shore just above the water's edge, making the sand green at many places.

The following algae were found in this lake:

Chroococcaceae
Clathrocystis aeruginosa
Coelosphaerium kuetzingianum
Merismopedium aerugineum
Oscillatoriaceae
Oscillatoria subtilissima
Phormidium tenue
Nostocaceae
Anabaena flos-aquae
Cylindrospermum majus
Nostoc austinii
Nostoc caeruleum
Nostoc glomeratum
Nostoc humifusum
Nostoc minutum
Nostoc muscorum
Nostoc pruniforme
Nostoc zetterstedtii
Rivulariaceae
Rivularia natans
Rivularia pisum
Bacillariaceae
Cocconeis placentula
Cymbella cymbiformis
Eunotia lunaris
Fragilaria capucina
Gomphonema acuminatum
Gomphonema parvulum
Navicula anglica
Navicula cuspidata
Navicula dicephala
Navicula sculpta
Navicula subcapitata
Navicula viridis
Nitzschia spectabilis
Nitzschia tryblionella
Surirella ovalis pinnata
Desmidiaceae
Closterium lanceolatum
Closterium leibleinii
Closterium moniliferum
Closterium pritchardianum
Cosmarium elfingii
Cosmarium formosulum nathrostii
Cosmarium granatum
Cosmarium kjellmani grande
Cosmarium laeve
Cosmarium obtusatum
Cosmarium retusiforme
Cosmarium subcrenatum
Cosmarium vexatum
Euastrum oblongum
Netrium digitus
Spirotaenia obscura
Staurastrum gracile
Tetrasporaceae
Dictyosphaerium pulchellum
Pleurococcaceae
Scenedesmus bijugatus
Scenedesmus obliquus
Scenedesmus quadricauda
Tetraedron trigonum
Urococcus insignis
Protococcaceae
Gloeocystis gigas
Gloeocystis vesiculosa
Hydrodictyaceae
Pediastrum boryanum
Pediastrum tetras
Ulotrichaceae
Microspora amoena
Chaetophoraceae
Chaetophora cornu-damae
Chaetophora elegans
Gongrosira debaryana
Oedogoniaceae
Bulbochaete sp.
Oedogonium fragile
Coleochaetaceae
Coleochaete orbicularis
Characeae
Chara contraria

CLEAR LAKE

This lake (Fig. 1, 2, 13) is a little wider and deeper than Hackberry lake and is about two-thirds of a mile distant from it. The water, however, is very alkaline (see discussion of alkalinity) and of a characteristic yellowish color. About the edge in places were rushes but there was little other vegetation in the lake. The algal flora was scarce in species but rich in individuals. Throughout the season the water was full of *Closterium aciculare*.[1] With this were found a few specimens of *Cosmarium obtusatum* and *Pediastrum boryanum*, and a few diatoms in fair abundance but that was all. Since in Clear lake the algae were all free floating, and the species were limited, no distribution studies were attempted. The same species were found throughout the summer. *Closterium aciculare* was the only characteristic form and was present throughout the season. The few other forms present with it were characteristic of the springs flowing into the lake and probably all came there by chance. It is probable that they could not have continued to live in so alkaline a water. Only one factor, alkalinity, could have influenced the algal flora of this lake.

In some places on the shore of the lake were springs whose water contained the richest algal flora found in the lake region. This was especially true at the west end (x in Fig. 13). Here the springs were on the sloping bank and the water seeped slowly down through the boggy soil. The surface was covered with grass and ferns forming sod enough to nearly support a hundred weight or more. Cattle coming to the spring for water had tramped over this sod forming holes from the size of a footprint to two or three feet in diameter. In these holes (Fig. 14) the water stood undisturbed for long periods with the thick grass sheltering the surface from the wind. The result was a large number of small aquaria. The water in these was kept uniformly fresh by seepage from the springs. While no chemical analysis was made, it was noticeable that the alkalinity was low.

[1] This form in size fits the description of the species as given by West and West (51) but may be the form referred to by West (50) as *Closteriopsis longissima* tho some of the specimens were over 600μ long and 7μ wide. He suggests that *Closteriopsis* may be a degenerate of *Closterium aciculare* var. *subpronum*.

The temperature was fairly uniform throughout the summer period varying from about 68° F. in the early morning to about 80° F. at 3 P.M. So far as was observed the ecological conditions in the various pockets were remarkably uniform in every way. Each was dominated by some one species, the usual dominants being *Anabaena torulosa*, various species of Nostoc, Spirogyra, Scytonema, Oedogonium, and Mougeotia. With these were mixed in various proportions the other species found. Attempts were made to determine whether there was any uniformity as to the species present with a given dominant but there seemed to be none.

The only ecological groupings of forms observed were seasonal. As will be seen from the accompanying list some forms were found only early in the summer, others late in the summer, and still others in the fall. The greatest variety of species was found in the middle of the summer. In the case of unicellular forms this list can only be looked upon as suggestive. Only a few specimens are found for some of them and chance in collecting may have caused others to be overlooked in one period or another. In this table early summer means from the middle of June to the middle of July; midsummer from the middle of July to the middle of August; fall is represented by collections made in October. Here fresh water, uniform temperature, protection from wind, and very shallow water form a habitat as uniform in every respect as is possible. Here the dominant forms found in a given water pocket can be due only to one of two things, seasonal periodicity which was very evident and the chance dominance of certain forms. Often the soil beneath a mass of Spirogyra, Mougeotia, or other such form was covered with desmids or diatoms. It is apparent that smaller forms, especially unicellular ones, may be shaded by the dominant species and hence their presence would be determined by their light relation. However, which of these shade loving forms should be associated with a given dominant species was chance so far as was determined. At other places on the lake shore were springs where a few algae were found but they were of so little consequence that the species were included only in the general list of species.

Clear Lake Puddles

	Early summer	Mid-summer	October
Chroococcaceae			
Aphanothece prasina	.....	x	.....
Clathrocystis aeruginosa	.....	x	.....
Gloeocapsa arenaria	.....	x	.....
Oscillatoriaceae			
Oscillatoria formosa	.....	x	.....
Oscillatoria limosa	x	.....	x
Phormidium tenue	.....	x	.....
Phormidium valderianum	.....	x	.....
Nostocaceae			
Anabaena flos-aquae	x	x	.....
Anabaena oscillarioides	.....	x	.....
Anabaena torulosa	x	x	x
Cylindrospermum comatum	.....	.....	x
Nodularia harveyana	.....	x	.....
Nostoc linckia	x	.....	x
Nostoc minutum	.....	x	.....
Nostoc muscorum	x	x	.....
Nostoc spongiaeforme	x	.....	.....
Scytonemaceae			
Scytonema crispum	x	x	x
Rivulariaceae			
Rivularia natans	.....	.....	x

*Bacillariaceae**	
Achnanthes lanceolata	Cystopleura zebra
Cyclotella meneghiniana	Eunotia diodon
Cymbella amphicephala	Eunotia lunaris
Cymbella cuspidata	Eunotia major
Cymbella naviculiformis	Fragilaria construens
Cymbella subaequalis	Gomphonema acuminatum
Cystopleura gibba	Gomphonema constrictum
Cystopleura gibba ventricosa	Gomphonema gracile
	Gomphonema montanum

* No seasonal studies were made of diatoms.

Melosira varians
Navicula ambigua
Navicula anglica
Navicula bacilliformis
Navicula brebissonii
Navicula cuspidata
Navicula dicephala
Navicula elliptica
Navicula gibba brevistriata
Navicula hilseana
Navicula major
Navicula mesolepta
Navicula iridis
Navicula pupula
Navicula sculpta
Navicula sphaerophora
Navicula stauroptera
Navicula viridis
Nitzschia brebissonii
Stauroneis anceps
Stauroneis anceps amphicephala
Stauroneis phoenicenteron
Surirella ovalis ovata
Synedra rumpens
Synedra ulna

	Early summer	Mid-summer	October
Desmidiaceae			
Arthrodesmus convergens	x	x	x
Closterium cynthia	x	x	x
Closterium didymotocum	x		
Closterium jenneri	x		
Closterium lanceolatum			x
Closterium lunula	x	x	x
Closterium moniliferum	x	x	x
Closterium parvulum		x	x
Closterium pritchardianum			x
Closterium siliqua			x
Closterium striolatum	x		
Closterium turgidum	x		
Cosmarium abruptum		x	
Cosmarium angulosum			x
Cosmarium angulosum concinnum		x	x
Cosmarium boeckii	x		x
Cosmarium blyttii		x	x
Cosmarium botrytis tumidum	x		
Cosmarium circulare	x		x
Cosmarium connatum	x	x	x
Cosmarium crenatum	x		
Cosmarium cyclicum	x		
Cosmarium cymatopleurum	x		
Cosmarium formosulum nathrostii		x	x
Cosmarium galeritum		x	
Cosmarium granatum		x	x

	Early summer	Mid-summer	October
Cosmarium holmiense	x		x
Cosmarium jkellmani grande		x	
Cosmarium meneghinii		x	
Cosmarium microsphinctum	x		
Cosmarium notabile	x		
Cosmarium obtusatum	x		
Cosmarium ochthodes		x	
Cosmarium pachydermum		x	x
Cosmarium pachydermum aethiopicum	x		x
Cosmarium phaseolus elevatum		x	
Cosmarium phaseolus forma minor	x		
Cosmarium portianum	x		x
Cosmarium pygmaeum	x		
Cosmarium pyramidatum		x	x
Cosmarium rectangulare hexagonum		x	x
Cosmarium sportella		x	
Cosmarium subcrenatum	x	x	
Cosmarium subtumidum		x	
Cosmarium subundulatum	x		
Cosmarium taxichondrum		x	
Cosmarium tetraophthalmum		x	
Cosmarium trilobulatum		x	
Cosmarium tumidum		x	
Cosmarium vexatum		x	
Euastrum attenuatum			x
Euastrum bidentatum		x	
Euastrum binale		x	x
Euastrum dubium		x	
Euastrum oblongum		x	x
Euastrum verrucosum	x	x	x
Euastrum verrucosum alatum		x	x
Micrasterias pinnatifida		x	x
Micrasterias rotata			x
Netrium digitus		x	x
Netrium interruptum			x
Penium libellula intermedium			x
Penium naegelii		x	
Penium spirostriolatum		x	x
Pleurotaenium coronatum	x	x	x
Pleurotaenium nodulosum		x	
Pleurotaenium trabeculata	x		

	Early summer	Mid-summer	October
Spirotaenia condensata	.....	x	x
Spirotaenia trabeculata	.....	x	.....
Staurastrum alternans	.....	.....	x
Staurastrum dickiei	.....	x	x
Staurastrum dilatatum	.....	.....	x
Staurastrum dispar	.....	x	.....
Staurastrum hirsutum	.....	x	x
Staurastrum margaritaceum	.....	x	x
Staurastrum meriani	.....	.....	x
Staurastrum muticum	.....	x	.....
Staurastrum orbiculare	x	x	x
Staurastrum orbiculare ralfsii	.....	x	.....
Staurastrum paxilliferum	.....	.....	x
Staurastrum polytrichum	x	.....	.....
Staurastrum punctulatum	x	x	x
Staurastrum saxonicum	.....	x	.....
Staurastrum teliferum	.....	x	.....
Zygnemaceae			
Spirogyra arcta catenaeformis	x	.....	.....
Spirogyra crassa	x	.....	.....
Spirogyra dubia	x	x	x
Spirogyra lutetiana	x	.....	.....
Spirogyra neglecta	.....	.....	x
Spirogyra varians	.....	.....	x
Zygnema sp	x	x	x
Mesocarpaceae			
Mougeotia robusta	.....	.....	x
Mougeotia scalaris	.....	.....	x
Mougeotia viridis	x	.....	.....
Volvocaceae			
Volvox aureus	.....	x	.....
Tetrasporaceae			
Dictyosphaerium pulchellum	.....	x	x
Tetraspora gelatinosa	x	.....	.....

	Early summer	Mid-summer	October
Pleurococcaceae			
Crucigenia rectangularis			x
Eremosphaera viridis		x	x
Nephrocytium naegelii	x		
Oocystis solitaria		x	x
Rhaphidium polymorphum falcatum		x	x
Scenedesmus antennatus	x		
Scenedesmus bijugatus	x		x
Tetraedron reticulatum			x
Urococcus insignis			x
Protococcaceae			
Gloeocystis vesiculosa	x		
Ophiocytium capitatum			x
Hydrodictyaceae			
Coelastrum sphaericum		x	
Pediastrum angulosum			x
Pediastrum boryanum	x	x	x
Pediastrum tetras		x	
Ulotrichaceae			
Microspora pachyderma		x	
Microspora stagnorum		x	
Chaetophoraceae			
Stigeoclonium glomeratum	x		
Oedogoniaceae			
Bulbochaete sp			x
Oedogonium sp	x	x	x
Oedogonium capilliforme australe		x	
Oedogonium cardiacum	x		
Oedogonium crispum uruguayense	x		
Oedogonium fragile		x	
Oedogonium varians			x
Cladophoraceae			
Rhizoclonium hieroglyphicum	x		

	Early summer	Mid-summer	October
Vaucheriaceae			
Vaucheria sp.			x

BIG ALKALI LAKE

Big Alkali Lake (Fig. 1) is a little larger than Clear lake. While it is more alkaline than Hackberry, Dewey, or Watts, it is far less so than Clear lake. The water had the characteristic yellow color of the alkaline lakes of this region. Here again alkalinity must be given as the factor governing the algal flora of the lake. One visit only was made here. At first sight the water seemed absolutely barren but further investigation showed the following forms to be present. The Chara was quite abundant forming very much dwarfed patches on the bottom near the shore.

Chroococcaceae
Clathrocystis aeruginosa
Merismopedium glaucum
Merismopedium tenuissimum
Oscillatoriaceae
Oscillatoria limosa
Bacillariaceae
Amphora ovalis
Campylodiscus clypeus
Cymbella cistula
Cystopleura gibba
Navicula cryptocephala veneta
Navicula gastrum
Navicula oblonga
Navicula sculpta
Desmidiaceae
Cosmarium angulosum
Cosmarium granatum
Cosmarium meneghinii
Cosmarium sexnotatum
Staurastrum gracile
Staurastrum paradoxum
Staurastrum polymorphum
Tetrasporaceae
Dictyosphaerium pulchellum
Pleurococcaceae
Scenedesmus bijugatus
Scenedesmus obliquus
Scenedesmus quadricauda
Hydrodictyaceae
Pediastrum boryanum
Pediastrum duplex clathratum
Characeae
Chara foetida rabenhorstii

DEWEY LAKE

Dewey lake (Fig. 1) is situated about half a mile from Hackberry lake. It is less alkaline, nearly three times as large and proportionally deeper. Otherwise the conditions were much the same. Temperatures taken in the region of algal growth showed little variation from those in Hackberry lake.

The algae were found along the margins of the lake and attached to submerged plants near the surface. As no attempt was made to study conditions in this lake no explanation of the conspicuous difference in the forms found can be given unless it was the larger amount of water and difference in alkalinity. Collections were made here at irregular intervals and the list must not be looked upon as complete.

Chroococcaceae
Clathrocystis aeruginosa
Merismopedium glaucum

Oscillatoriaceae
Beggiatoa alba
Lyngbya aerugineo-caerulea
Oscillatoria amphibia
Oscillatoria formosa
Oscillatoria subtilissima
Phormidium fragile
Phormidium tenue
Phormidium valderianum

Nostocaceae
Anabaena flos-aquae
Nostoc linckia
Nostoc pruniforme

Scytonemaceae
Tolypothrix distorta

Rivulariaceae
Rivularia echinulata
Rivularia natans

Bacillariaceae
Amorpha ovalis
Brebissonia vulgaris
Cocconeis placentula
Cymbella cistula
Cymbella cuspidata
Cymbella lanceolata
Encyonema turgidum
Eunotia lunaris
Fragilaria capucina
Fragilaria construens binodis
Gomphonema constrictum
Gomphonema gracile
Gomphonema montanum
Gomphonema parvulum
Navicula cuspidata
Navicula lanceolata
Navicula major
Navicula oblonga
Navicula pupula
Staureneis acuta
Stauroneis smithii
Stauroneis tenuissima
Synedra rumpens
Synedra ulna

Desmidiaceae
Closterium pritchardianum
Cosmarium blyttii
Cosmarium boeckii
Cosmarium formosulum nathorstii
Cosmarium impressulum
Cosmarium obtusatum
Cosmarium ochthodes var.
Cosmarium subcrenatum
Cosmarium turpinii podolicum
Cosmarium vexatum
Penium margaritaceum
Staurastrum orbiculare

Pleurococcaceae
Scenedesmus bijugatus
Scenedesmus obliquus
Tetraedron trigonum

Protococcaceae
Characium ambiguum
Characium subulatum
Gloeocystis vesiculosa

Hydrodictyaceae
Pediastrum boryanum

Ulotrichaceae
Hormiscia subtilis variabilis

Chaetophoraceae
Chaetophora elegans
Gongrosira debaryana
Stigeoclonium aestivale

Stigeoclonium glomeratum

Oedogoniaceae

Oedogonium grande

Oedogonium vaucherii

Helminthocladiaceae

Batrachospermum vagum

WATTS LAKE

Watts lake (Fig. 1) is about half the size of Hackberry and only about one-third of a mile from it. Conditions in the lake were very similar to those in Hackberry in every respect. It was, however, slightly less alkaline than Dewey lake.

Collections were made here about once a week but other data were not taken. No boat was available on this lake and that may account for some of the difference in the reports for Watts and Hackberry lakes. No reason for a difference in species could be given unless it were the slight difference in alkalinity. The following species were found which it will be noted are nearly all included in those found in Hackberry and Dewey lakes and the springs on the shore of Clear lake.

Chroococcaceae

Clathrocystis aeruginosa
Coelosphaerium kuetzingianum
Merismopedium tenuissimum
Microcystis marginata

Nostocaceae

Nostoc pruniforme

Bacillariaceae

Achnanthes lanceolata
Amphora ovalis
Cocconeis placentula
Gomphonema gracile
Gomphonema montanum
Homoeocladia amphioxys
Navicula gastrum
Navicula lanceolata

Desmidiaceae

Cosmarium boeckii
Cosmarium formosulum nathorstii
Cosmarium geminatum
Cosmarium granatum
Cosmarium obtusatum
Cosmarium phaseolus
Cosmarium pseudopyramidatum
Cosmarium subcrenatum
Staurastrum gracile
Staurastrum margaritaceum

Tetrasporaceae

Dictyosphaerium pulchellum

Pleurococcaceae

Oocystis solitaria
Scenedesmus bijugatus
Scenedesmus obliquus
Scenedesmus quadricauda
Tetraedron minimum

Hydrodictyaceae

Pediastrum boryanum

Characeae

Chara contraria

OTHER LAKES

One visit was made to each of the following lakes in the early summer. Trout and Dad's lakes (Fig. 1) are among the larger of the

group while Phalaris (Fig. 1) is one of the smaller. The Snake Creek Falls, about 15 miles distant, were visited once. These are falls in a creek which flows through the sandhill region. These collections were made so superficially that the lists must stand only as representing some species found in these localities.

The only form found which was sufficiently conspicuous to need special mention was *Nostoc verrucosum* which was extremely abundant on rocks in the cataract below the falls in Snake Creek.

The forms found in these localities are as follows:

PHALARIS LAKE

Chroococcaceae

Coelosphaerium kuetzingianum
Merismopedium tenuissimum
Dactylococcopsis rhaphidioides

Bacillariaceae

Amphora ovalis
Cocconeis placentula
Cystopleura gibba
Cystopleura turgida
Gomphonema montanum
Homoeocladia amphibia
Navicula cryptocephala veneta
Navicula cuspidata
Navicula elliptica
Navicula gastrum
Navicula oblonga
Navicula sculpta
Navicula sphaerophora
Sceptroneis fibula

Desmidiaceae

Cosmarium angulosum
Cosmarium blyttii
Cosmarium formosulum nathorstii
Cosmarium granatum subgranatum
Cosmarium obtusatum
Cosmarium regnellii

Tetrasporaceae

Dictyosphaerium pulchellum

Pleurococcaceae

Oocystis solitaria
Pediastrum boryanum
Scenedesmus bijugatus
Scenedesmus quadricauda
Tetraedron minimum

Characeae

Chara contraria
Chara evoluta
Chara fragilis
Chara sp.

TROUT LAKE

Chroococcaceae

Clathrocystis aeruginosa

Nostocaceae

Anabaena flos-aquae
Nostoc zetterstedtii

Hydrodictyaceae

Pediastrum angulosum

Characeae

Chara sp.

DAD'S LAKE

Chroococcaceae

Clathrocystis aeruginosa

Desmidiaceae

Closterium acerosum

Tetrasporaceae

Dictyosphaerium pulchellum

Hydrodictyaceae

Pediastrum boryanum
Pediastrum duplex clathratum

SNAKE FALLS

Chroococcaceae
Merismopedium glaucum
Oscillatoriaceae
Arthrospira jenneri
Oscillatoria brevis
Oscillatoria formosa
Phormidium retzii
Nostocaceae
Nostoc pruniforme
Nostoc verrucosum
Rivulariaceae
Calothrix parietina
Bacillariaceae
Achnanthes lanceolata
Amphora ovalis
Cocconeis placentula
Cymbella amphicephala
Cymbella cuspidata
Cymbella ehrenbergii
Cymbella gastroides
Cystopleura gibba
Cystopleura ocellata
Cystopleura turgida
Cystopleura zebra
Denticula elegans
Encyonema turgidum
Eunotia major
Fragilaria mutabilis
Gomphonema acuminatum
Gomphonema herculeanum
Lysigonium crenulatum
Lysigonium distans
Navicula ambigua
Navicula anglica
Navicula appendiculata
Navicula brebissonii
Navicula cuspidata craticula
Navicula dicephala
Navicula elliptica
Navicula humilis
Navicula iridis
Navicula lanceolata
Navicula limosa
Navicula gibba brevistriata
Navicula mesolepta
Navicula pupula
Navicula radiosa
Navicula sculpta
Navicula viridis
Homoeocladia amphibia
Homoeocladia brebissonii
Homoeocladia amphioxys
Homoeocladia palea
Rhoicosphenia curvata
Sceptroneis pacifica
Stauroneis anceps
Stauroneis phoenicenteron
Surirella robusta
Surirella spiralis
Synedra ulna
Tetracyclus lacustris
Desmidiaceae
Closterium striolatum
Cosmarium microsphinctum
Cosmarium portianum
Cosmarium sportella
Cosmarium undulatum wollei
Euastrum oblongum
Euastrum verrucosum
Penium margaritaceum
Staurastrum orbiculare hibernicum
Pleurococcaceae
Scenedesmus obliquus
Protococcaceae
Chlorococcum humicola
Cladophoraceae
Cladophora glomerata

CONCLUSION

It appears even from so brief a study as the one just described that the occurrence of algae in a given body of water at a given time is

due, to a certain extent, as Transeau (44), West (49), and others have said, to seasonal periodicity.

It is also evident that West (48 and 50), Oltmanns (34), Brannon (12), Wipple and Parker (53), Chambers (15), and many others are correct in their decisions that the mineral and gas content of water has much to do with its algal flora. Of these factors, alkalinity is probably to a great extent, the explanation for the wide difference in the algal flora of lakes so close together and so uniform in all other factors.

In a given lake the distribution of species may be explained by the one factor only that is variable, namely light intensity. Means for measuring this factor were entirely inefficient and only the crudest estimates can be made.

In small bodies of water where even the light is not variable to any measurable degree the dominant species and its associates are determined merely by chance except that forms lying beneath other forms are more shaded. This exception does not affect the dominant species but may affect the forms associated with it.

ALGAE FOUND IN CHERRY COUNTY

Chroccoccaceae.
- Aphanothece prasina A. Braun
- Clathrocystis aeruginosa (Kuetz.) Henfrey
- Coelosphaerium kuetzingianum Naeg.
- Dactylococcopsis rhaphidioides Hansg.
- Gloeocapsa arenaria (Hassall) Rabenh.
- Merismopedium aerugineum Bréb.
- Merismopedium glaucum (Ehrb.) Naeg.
- Merismopedium tenuissimum Lemmerm.
- Microcystis marginata (Menegh.) Kuetz.

Oscillatoriaceae.
- Arthrospira jenneri (Kuetz.) Stiz.
- Lyngbya aerugineo-caerulea (Kuetz.) Gom.
- Oscillatoria amphibia Ag.
- Oscillatoria brevis (Kuetz.) Gom.
- Oscillatoria formosa Bory.
- Oscillatoria limosa. (Roth) Ag.
- Oscillatoria princeps Vauch.
- Oscillatoria sancta (Kuetz.) Gom.
- Oscillatoria subtilissima Kuetz.
- Oscillatoria tenuis Ag.
- Phormidium fragile (Menegh.) Gom.
- Phormidium retzii (Ag.) Gom.
- Phormidium tenue (Menegh.) Gom.
- Phormidium valderianum (Delp.) Gom.
- Spirulina major Kuetz.

Nostocaceae.
- Anabaena flos-aquae (Lyngb.) Bréb.
- Anabaena oscillarioides Bory.
- Anabaena torulosa (Carmich.) Lagerheim
- Cylindrospermum comatum Wood
- Cylindrospermum majus Kuetz.
- Nodularia harveyana(Thwaites)Thuret
- Nostoc austini Wood
- Nostoc caeruleum Lyngbye

Nostoc commune Vaucher
Nostoc glomeratum Kuetz.
Nostoc humifusum Carmichael
Nostoc linckia (Roth) Bornet
Nostoc minutum Desm.
Nostoc muscorum Ag.
Nostoc pruniforme (L.) Ag.
Nostoc spongiaeforme Ag.
Nostoc verrucosum (L.) Vauch.
Nostoc zetterstedtii Areschoug
Scytonemaceae.
Scytonema crispum (Ag.) Bornet
Tolypothrix distorta (Hofm. B.) Kuetz.
Rivulariaceae.
Calothrix parietina (Naeg.) Thur.
Rivularia echinulata (Smith) Born
Rivularia natans (Hedw.) Welw.
Rivularia pisum Ag.
Bacillariaceae.
Achnanthes lanceolata (Bréb.) Gr.
Amorpha ovalis (Bréb.) Kuetz.
Brebissonia vulgaris (Thwait) Kunze
Campylodiscus clypeus Ehr.
Cocconeis placentula Ehr.
Cyclotella meneghiniana Kuetz.
Cymbella amphicephala Naeg.
Cymbella cistula (Hempr.) Kirchn.
Cymbella cuspidata Kuetz.
Cymbella cymbiformis (Kuetz.) Bréb.
Cymbella ehrenbergii Kuetz.
Cymbella lanceolata (Ehr.) Kirch.
Cymbella naviculiformis Auersw.
Cymbella subaequalis Grun.
Cystopleura gibba (Ehr.) Kunze
Cystopleura zebra (Ehr.) Kunze
Encyonema turgidum (Greg.) Grun.
Eunotia diodon Ehr.
Eunotia lunaris Grun.
Eunotia major (W. Sm.) Rabenh.
Fragilaria capucina Desmaz.
Fragilaria construens (Ehr.) Grun.
Fragilaria construens binodis (Ehr.) Grun.
Gomphonema acuminatum Ehr.
Gomphonema constrictum Ehr.
Gomphonema gracile Ehr.
Gomphonema herculeanum Ehr.
Gomphonema montanum Schum.
Gomphonema parvulum Kuetz.
Homoeocladia amphibia (Grun.) Kunze
Homoeocladia amphioxys (Ehr.) Kunze
Homoecladia brebissonii (H. Sm.) Kunze
Homoeocladia palea (Kuetz.) Kunze
Lysigonium crenulatum (Kuetz.)Kunze
Lysigonium distans (Kuetz.) Kunze
Lysigonium varians (Ag.) D.T.
Navicula ambigua Ehr.
Navicula anglica Ralfs
Navicula appendiculata (Ag.) Kuetz.
Navicula bacilliformis Grun.
Navicula brebissonii Kuetz.
Navicula cryptocephala veneta (Kuetz.) Rhabenh.
Navicula cuspidata Kuetz.
Navicula dicephala Ehr.
Navicula elliptica Kuetz.
Navicula gastrum Ehr.
Navicula gibba (Ehr.) Kuetz.
Navicula gibba brevistriata Grim.
Navicula hilseana Jan.
Navicula humilis Donk.
Navicula iridis Ehr.
Navicula lanceolata Kuetz.
Navicula limosa Kuetz.
Navicula major Kuetz.
Navicula mesolepta Ehr.
Navicula oblonga Kuetz.
Navicula pupula Kuetz.
Navicula radiosa Kuetz.
Navicula sculpta Ehr.
Navicula sphaerophora Kuetz.
Navicula stauroptera Grun.
Navicula subcapitata Greg.
Navicula viridis Kuetz.
Nitzschia brebissonii W. Sm.
Nitzschia spectabilis (Ehr.) Ralfs
Nitzschia tryblionella Hantzsch.
Rhoicosphenia curvata Grun.
Sceptroneis fibula (Bréb.) Schuett

Sceptroneis pacifica (Grun.) Elmore (In press)
Stauroneis anceps Ehr.
Stauroneis anceps amphicephala Kuetz.
Stauroneis acuta W. Sm.
Stauroneis phoenicenteron Ehr.
Stauroneis smithii Grun.
Surirella ovalis ovata (Bréb.) V.H.
Surirella ovalis pinnata (Bréb.) V.H.
Surirella robusta Ehr.
Surirella spiralis Kuetz.
Synedra rumpens Kuetz.
Synedra ulna (Nitzsch.) Ehr.
Tetracyclus lacustris Ralfs

Desmidiaceae.

Arthrodesmus convergens Ehrenb.
Closterium acerosum (Schrank) Ehrenb.
Closterium aciculare Tuffen West
Closterium cynthia DeNot.
Closterium didymotocum Corda
Closterium jenneri Ralfs
Closterium lanceolatum Kuetz.
Closterium leibleinii Kuetz.
Closterium lunula (Muell.) Nitzsch.
Closterium moniliferum (Bory). Ehrenb.
Closterium parvulum Naeg.
Closterium pritchardianum Arch.
Closterium siliqua West and G. S. West
Closterium striolatum Ehrenb.
Closterium turgidum Ehrenb.
Cosmarium abruptum Lund.
Cosmarium angulosum Bréb.
Cosmarium angulosum concinnum (Rabenh.) West and G. S. West
Cosmarium blyttii Wille
Cosmarium boeckii Wille
Cosmarium botrytis tumidum Wolle
Cosmarium circulare Reinsch.
Cosmarium connatum Bréb.
Cosmarium crenatum Ralfs
Cosmarium cyclicum Lund.
Cosmarium cymatopleurum Nordst.
Cosmarium elfingii Racib.
Cosmarium formosulum nathorstii (Boldt) West and G. S. West
Cosmarium galeritum Nordst.
Cosmarium geminatum Lund.
Cosmarium granatum Bréb.
Cosmarium granatum subgranatum Nordst.
Cosmarium holmiense Lund.
Cosmarium holmiense integrum Lund.
Cosmarium impressulum Elfv.
Cosmarium kjellmani grande Wille
Cosmarium laeve Rabenh.
Cosmarium meneghinii Bréb.
Cosmarium microsphinctum Nordst.
Cosmarium notabile Bréb.
Cosmarium obtusatum Schmidle
Cosmarium ochthodes Nordst. var.
Cosmarium pachydermum Lund.
Cosmarium pachydermum aethiopicum West and G. S. West
Cosmarium phaseolus Bréb.
Cosmarium phaseolus elevatum Nordst.
Cosmarium phaseolus minor Boldt
Cosmarium portianum Arch.
Cosmarium protractum (Naeg.) De-Bary
Cosmarium pseudopyramidatum Lund.
Cosmarium pygmaeum Arch.
Cosmarium pyramidatum Bréb.
Cosmarium rectangulare hexagonum (Elf.) West and G. S. West
Cosmarium regnellii Wille
Cosmarium retusiforme (Wille) Gutw.
Cosmarium sexnotatum Gutw.
Cosmarium sportella Bréb.
Cosmarium subcrenatum Hantzsch
Cosmarium subtumidum Nordst.
Cosmarium subundulatum Wille
Cosmarium taxichondrum Lund.
Cosmarium tetraophthalmum Bréb.
Cosmarium trilobulatum Reinsch
Cosmarium tumidum Lund.
Cosmarium turpinii podolicum Gutw.
Cosmarium umbilicatum Luetkem.

Cosmarium undulatum wollei West
Cosmarium vexatum West
Euastrum attenuatum Wolle
Euastrum bidentatum Naeg.
Euastrum binale (Turp.) Ehrenb.
Euastrum dubium Naeg.
Euastrum oblongum (Grev.) Ralfs
Euastrum verrucosum Ehrenb.
Euastrum verrucosum alatum Wolle
Micrasterias pinnatifida (Kuetz.) Ralfs
Micrasterias rotata (Grev.) Ralfs
Netrium digitus (Ehrenb.) Itzigs and Rothe
Netrium interruptum (Bréb.) Luetkem.
Pleurotaenium coronatum (Bréb.) Rabenh.
Penium libellula (Focke) Nordst.
Penium margaritaceum (Ehrenb.) Bréb.
Penium naegelii Bréb.
Penium spirostriolatum Barker
Pleurotaenium nodulosum (Bréb.) De-Bary
Pleurotaenium trabecula (Ehrenb.) Naeg.
Spirotaenia condensata Bréb.
Spirotaenia obscura Ralfs
Spirotaenia trabeculata A. Br.
Staurastrum alternans Bréb.
Staurastrum dickiei Ralfs
Staurastrum dilatatum Ehrenb.
Staurastrum dispar Bréb.
Staurastrum gracile Ralfs
Staurastrum hirsutum (Ehrenb.) Bréb.
Staurastrum margaritaceum Ehrenb.
Staurastrum meriani Reinsch
Staurastrum muticum Bréb.
Staurastrum orbiculare (Ehrenb.) Ralfs
Staurastrum orbiculare hibernicum West and G. S. West
Staurastrum orbiculare ralfsii West and G. S. West
Staurastrum paradoxum Meyen
Staurastrum paxilliferum G. S. West
Staurastrum polymorphum Bréb.
Staurastrum punctulatum Bréb.
Staurastrum saxonicum Bulnh.
Staurastrum teliferum Ralfs

Zygnemaceae.
Spirogyra arcta catenaeformis Kirchn.
Spirogyra crassa Kuetz.
Spirogyra dubia Kuetz.
Spirogyra lutetiana Petit
Spirogyra neglecta (Hass.) Kuetz.
Spirogyra varians (Hass.) Kuetz.
Zygnema Ag. sp.

Mesocarpaceae.
Mougeotia robusta (DeBary) Wittr.
Mougeotia scalaris Hass.
Mougeotia viridis (Kuetz.) Wittr.

Volvocaceae.
Volvox aureus Ehrenb.

Tetrasporaceae.
Tetraspora gelatinosa (Vauch.) Desv.
Dictyosphaerium pulchellum Wood

Pleurococcaceae.
Crucigenia rectangularis (A. Br.) Gay
Eremosphaera viridis DeBary
Nephrocytium naegelii Grun.
Oocystis solitaria Wittr.
Rhaphidium polymorphum falcatum (Corda) Rabenh.
Scenedesmus antennatus Bréb.
Scenedesmus bijugatus (Turp.) Kuetz.
Scenedesmus obliquus (Turp.) Kuetz.
Scenedesmus quadricauda (Turp.) Bréb.
Tetraedron minimum Reinsch
Tetraedron reticulatum (Reinsch) Hansg.
Tetraedron trigonum (Naeg.) Hansg.
Urococcus insignis Hass.

Protococcaceae.
Characium ambiguum Hermann
Chlorococcum humicola (Naeg.) Rabenh.
Characium subulatum A. Br.
Gloeocystis gigas (Kuetz.) Lagerh.
Gloeocystis vesiculosa Naeg.
Ophiocytium capitatum Wolle

Hydrodictyaceae.
- Coelastrum sphaericum Naeg.
- Pediastrum angulosum (Ehrenb.) Menegh.
- Pediastrum boryanum (Turp.) Menegh.
- Pediastrum duplex clathratum A. Br.
- Pediastrum tetras (Ehrenb.) Ralfs

Ulotrichaceae
- Microspora amoena (Kuetz.) Rabenh.
- Microspora pachyderma (Wille) Lagerh.
- Microspora stagnorum (Kuetz.)Lagerh.
- Hormiscia subtilis variabilis (Kuetz.) Kirchn.

Chaetophoraceae.
- Chaetophora cornu-damae (Roth) Ag.
- Chaetophora elegans (Roth) Ag.
- Gongrosira debaryana Rabenhorst
- Stigeoclonium aestivale (Hazen) Collins
- Stigeoclonium glomeratum (Hazen) Collins

Oedogoniaceae.
- Bulbochaete Ag. sp.
- Oedogonium Link (several species not in fruit.)
- Oedogonium capilliforme australe Wittr.
- Oedogonium cardiacum (Hass.) Kuetz.
- Oedogonium crispum uruguayense Magn. and Wille
- Oedogonium fragile Wittr.
- Oedogonium grande Kuetz.
- Oedogonium varians Wittr. and Lund.
- Oedogonium vaucherii (LeCl.) A. Br.

Coleochaetaceae.
- Coleochaete orbicularis Pringsh.

Cladophoraceae.
- Cladophora glomerata (L.) Kuetz.
- Rhizoclonium hieroglyphicum (Ag.) Kuetz.

Vaucheriaceae.
- Vaucheria D.C. sp.

Characeae.
- Chara Vaill. sp.
- Chara contraria A. Br.
- Chara evoluta Allen
- Chara foetida rabenhorstii T. F. Allen
- Chara fragilis Desv.

Helminthocladiaceae.
- Batrachospermum vagum Ag.

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EXPLANATION OF FIGURES

Fig. 1. Map showing the lake region in Cherry County, Nebraska. Clear white areas represent water, dotted areas wet meadows, and ||||||||| swamps. (From map by Dr. G. E. Condra).

Fig. 2. A view taken from the top of a sandhill and showing at the front left a part of Clear lake, at the upper left a part of Dewey lake and in the distance at the right a narrow strip of White Water lake, also the "sandhills" and the meadows surrounding the lakes. (Photo by F. H. Shoemaker).

Fig. 3. Hackberry lake from the northeast shore.

Fig. 4. Taking water photometer records among the rushes on Hackberry lake.

Fig. 5. Submerged moss stems covered with Nostoc glomeratum—Hackberry lake. (Photographed under water).

Fig. 6. Section of Scirpus stem covered with Chaetophora cornu-damae and Nostoc glomeratum. (Photographed under water).

Fig. 7. Water photometer records on solio paper. Upper row exposures made in air. Three lower lows exposures made under water.

Fig. 8. Thermographs and anemometers in a blowout near the shore of Hackberry lake.

Fig. 9. Thermographs and anemometers in the grassy meadow on the shore of Hackberry lake.

Fig. 10. Lower end of water photometer showing water tight drum and window covered with ray filter. (Photo by F. H. Shoemaker.)

Fig. 11. Upper end of water photometer showing lever by means of which successive areas of the photographic plate may be exposed to the window. (Photo by F. H. Shoemaker.)

Fig. 12. Above, under side of upper half of drum showing perforated, revolving disk to which photographic plates are attached by means of two clips. (Photo by F. H. Shoemaker.)

Fig. 12. Below, upper half of water tight drum with lower half and tube removed. (Photo by F. H. Shoemaker.)

Fig. 13. Clear lake from a sandhill at its southwest end. At the right in the distance a narrow strip of Willow lake. (X) the location of springs shown in fig. 14.

Fig. 14. Pockets of spring water on the southwest shore of Clear lake.

Fig. 15. Chart showing: A, temperature of air; B, temperature of soil 8 inches below surface; and C, temperature of surface soil at a station in a blowout near the shore of Hackberry lake. Also D, temperature of air in shade of a building on the lake shore; E, wind velocity at the rim of the blowout; and F, wind velocity at the bottom of the same blowout. Numbers at the left indicate, above temperature in Fahrenheit and below miles per hour of wind velocity.

Fig. 16. Chart showing: A, temperature of air; B, temperature of soil eight inches below the surface; and C, temperature of surface soil at a station in the grass near the lake shore; also D, temperature of air in the shade of a building on the lake shore; and E, wind velocity for the same period. Numbers at left indicate, above temperature in Fahrenheit and below miles per hour of wind velocity at the station.

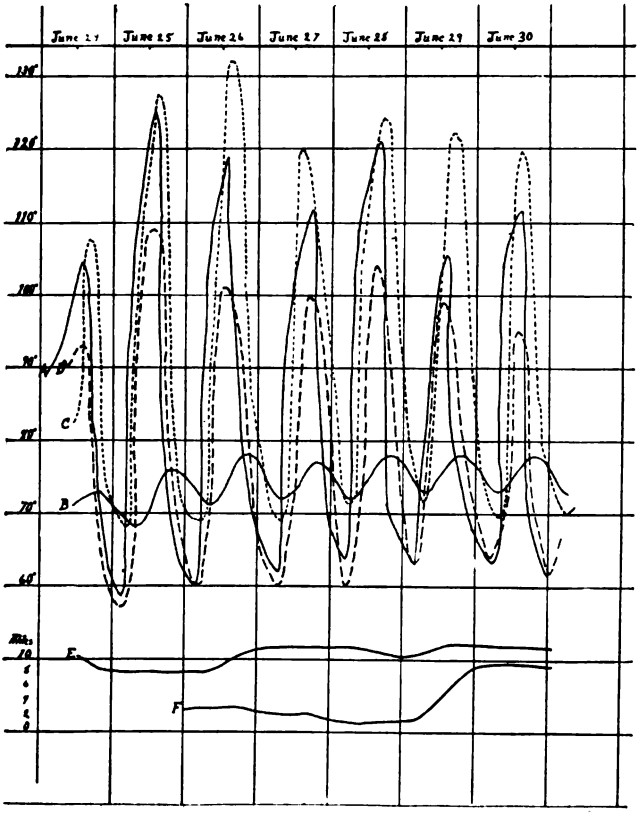


PLATE X Fig. 15. ANDERSEN AND WALKER

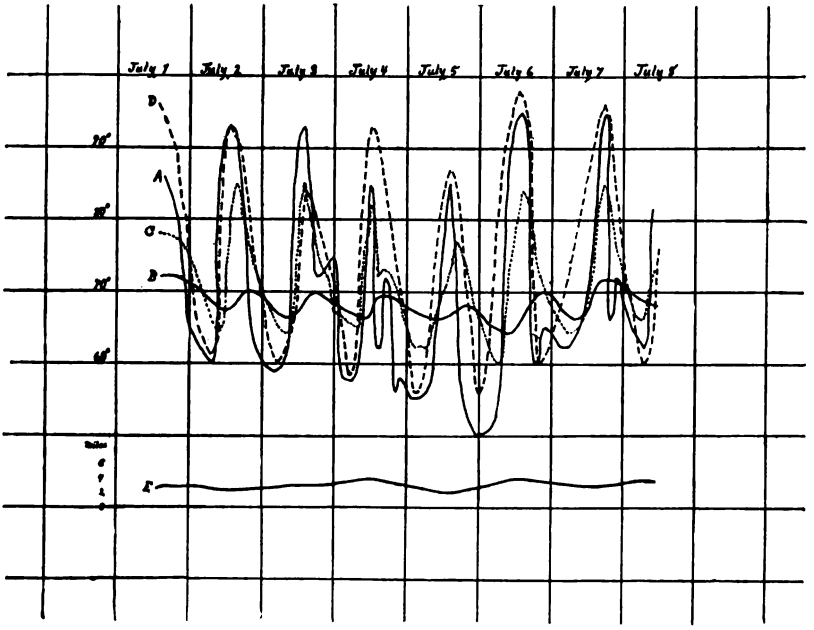
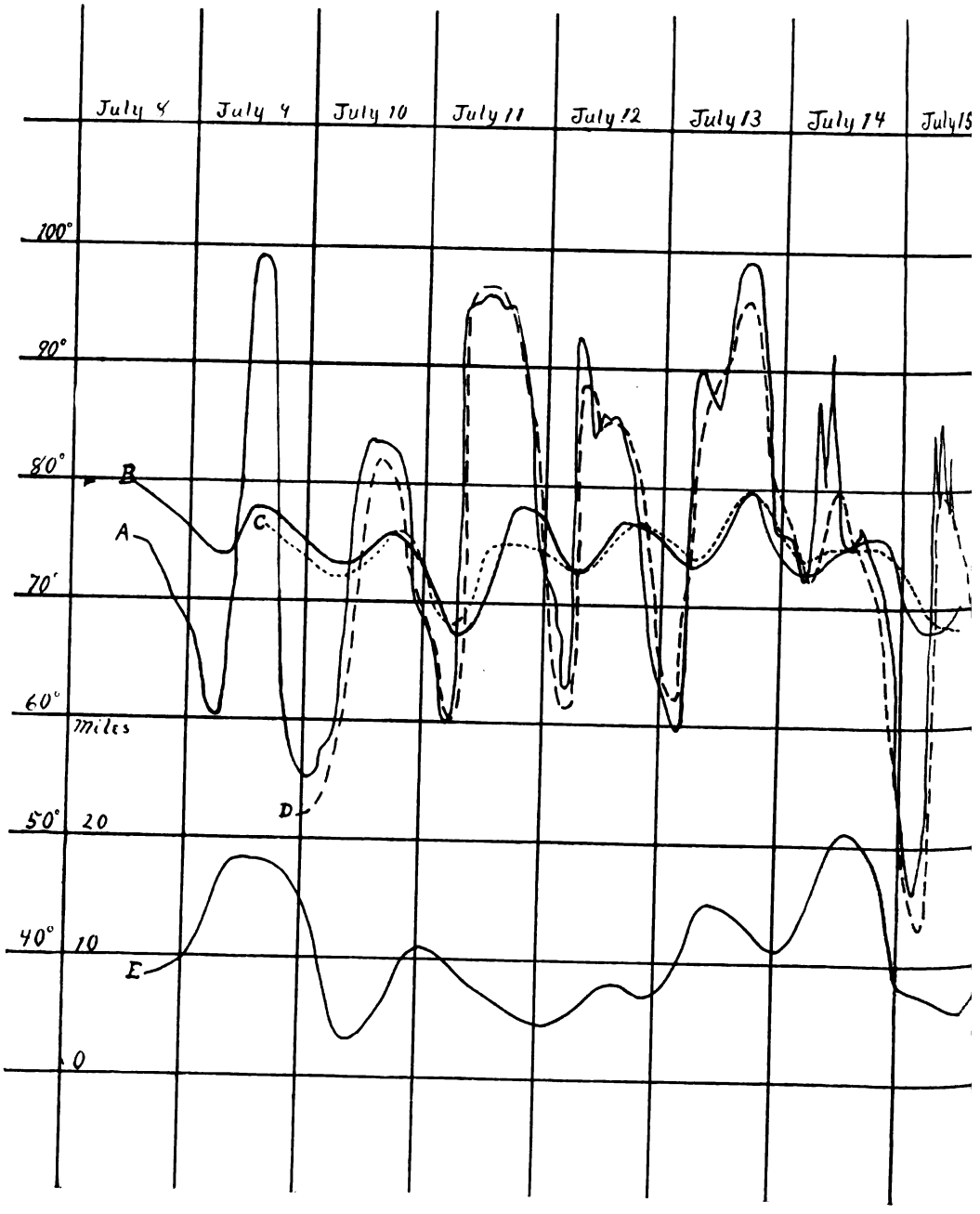
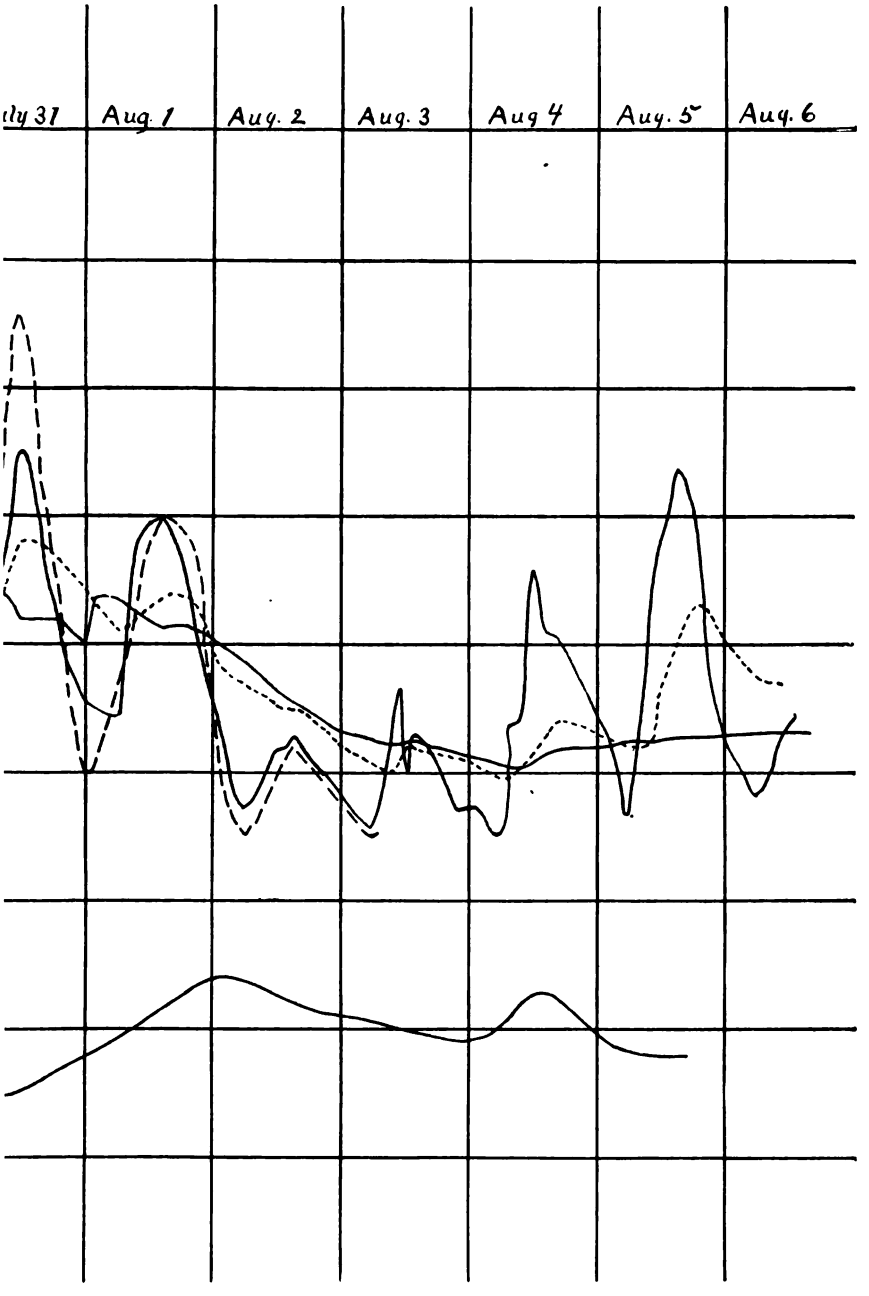


PLATE XI Fig. 16. ANDERSEN AND WALKER





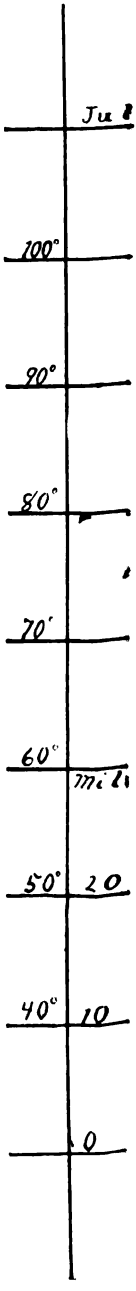


Fig. 17. Chart showing: A, air temperature; B, temperature of water at the bottom of lake (3 feet below surface the first week, 4½ feet below surface the second and third week, and 5 feet below surface the fourth week); C, temperature of water at surface of lake at a station located in a boat anchored in the lake; D, temperature of air in shade of a building on the lake shore; E, wind velocity at margin of lake. Numbers at left indicate above temperature in Fahrenheit and below miles per hour of wind velocity. July 24–27 anemometer readings were not taken. It was a period of very low wind and is indicated approximately by the dotted line.

The University of Nebraska,
Lincoln, Nebr.

DEPARTMENT OF NOTES AND REVIEWS

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In addition to these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

LEECHES CONSIDERED AS OLIGOCHAETA MODIFIED FOR A PREDATORY LIFE

Michaelsen (Mitt. Zool. Mus. XXXVI, Hamburg, 1919) was led to a study of the relationships between these two groups of animals, by noticing a figure in a recent paper on Sudanese Hirudinea. The figure represented an organ that was interpreted by the author, as a diverticulum of the alimentary tract of the leech, opening to the exterior on the mid-dorsal surface of the 13th somite. Similar organs have been described in certain leeches from Sumatra, in which they are paired, and the external pores are ventrally situated. The figured organ strongly resembles the spermathecae of certain oligochaete species in the families of Enchytraeidae and Lumbriculidae, in which the spermathecae communicate internally with the alimentary tract. Similar relations have also been found in certain species of other families of Oligochaeta.

As a result of his studies, Michaelsen has reached the conclusion that the Hirudinea are, in reality, Lumbriculidae which have undergone special modifications in adaptation to a predatory mode of life. He believes that such a conclusion receives much support from a careful comparison of the structure of two intermediate types of worms: the Branchiobdellidae, and *Acanthobdella peledina* Grube. The former are parasitic in the gill chambers and on parts of the surface of crawfishes, and, as their name indicates, were formerly included with leeches; but recently their closer relationship with the Oligochaeta is generally admitted. *Acanthobdella peledina* is a peculiar leech-like parasite of certain fishes of the genus Salmo, in northeastern Europe, and in western Siberia. On the ventral surface

of several anterior somites, are paired bundles of setae, and the characters of the reproductive organs and of the body cavity are also nearer to those of the Oligochaeta than to those of the leeches. Michaelsen concludes that, although there is some justification for including these two groups in the family Lumbriculidae, it is nevertheless preferable to recognize them as two distinct families of Oligochaeta, Branchiobdellidae and Acanthobdellidae closely related to the Lumbriculidae. After making this disposition of these two groups, the author makes a comparison of the various structural characters of the Hirudinea and Oligochaeta.

Attention is called to the fact that there is a wide range of variation among different representatives of the Oligochaeta, and that most of the characters which one is accustomed to think of as typical of the Oligochaeta are not present in all members of the group, though they may be in a majority of the better known ones. It is also shown that many of the characters of Hirudinea which one is likely to assume as distinguishing them from Oligochaeta, may be found present in certain members of the latter group. Absence of setae occurs in a genus of the oligochaete family Enchytraeidae, as well as in Branchiobdellidae, and they are greatly reduced in numbers and size in various other representatives. As previously mentioned, four pairs of well developed setae are present on each of several anterior somites in *Acanthobdella peledina* which has previously, without question, been assumed to belong to the Hirudinea. The shortened body and thickened body wall of the leeches, with a correlated reduction of the body cavity, are already forecast in Chaetogaster and in certain species of Lumbriculidae, to say nothing of the Branchiobdellidae and Acanthobdella. They are natural accompaniments of a change of food, and assumption of a predatory mode of life.

There is great variation in the structure of the nephridia among the Oligochaeta, and absence of ciliated nephrostomes and of cilia in the excretory part of the ducts is found in species of diverse groups. The ventro-median position of the pores of the efferent ducts of the reproductive organs of leeches has a counterpart in certain species of Lumbriculidae and of the earthworm subfamily Eudrilinae.

The most significant character which distinguishes the Hirudinea, in general, from the Oligochaeta, is the position of the spermaries

in somites posterior to the one which contains the ovaries. This relative position of the two kinds of gonads is the opposite of that normally found in Oligochaeta, and in the connecting forms, Branchiobdellidae and Acanthobdella. To account for this reversal of relations, the author refers to instances where Oligochaeta are found with a considerable number of consecutive somites containing gonads; and also to papers by different writers, in which gonads of certain oligochaete species have been shown to produce one kind of germ cells at one time, and at other times to produce those of the opposite kind. From individuals with series of gonads of this type, he thinks it not improbable that there may have been derived descendants in which the relative position of the gonads of the two sexes is in the reverse order from that of the ancestors. For details of structure and references to the literature involved in these comparisons, the original paper must be consulted.

The author thinks it desirable to modify the outlines of classification, to fit these new views of relationship. He proposes a class Clitellata which is co-ordinate with the class Chaetopoda, and with three other classes which contain marine forms and are not involved. The class Clitellata includes two orders, Oligochaeta and Hirudinea; distinguished chiefly by the differences in the degree of development of the body cavity, and the relative order of the gonads. The class Chaetopoda includes two orders, Protochaeta and Polychaeta.

FRANK SMITH

Department of Zoology,
Univ. of Illinois

PROCEEDINGS OF THE AMERICAN MICROSCOPICAL SOCIETY

Minutes of the St. Louis Meeting

The thirty-eighth annual meeting of the American Microscopical Society was held in affiliation with the A.A.A.S. at St. Louis, Mo., Dec. 31, 1919.

In the absence of President Griffin, Vice-President Whelpley acted as chairman.

The report of the Treasurer for the years 1918 and 1919 was accepted and referred to an auditing committee composed of Professors H. B. Ward and H. J. VanCleave.

The report of the Custodian was accepted, ordered printed, and referred to an auditing committee composed of Messrs. Edw. Pennock and Edw. P. Dolbey.

A vote of appreciation was extended to Professor T. W. Galloway, the retiring Secretary, for a most valuable service rendered to the Society during the past ten years.

The meeting voted approval of the action of the Executive Committee in appointing Mr. Wm. F. Henderson as Treasurer, and Professor Paul S. Welch as Secretary at dates in advance of the regular annual business meeting.

The following officers were duly nominated and elected for the constitutional periods: President, Professor T. W. Galloway, New York; First Vice-President, Chancey Juday, University of Wisconsin; Second Vice-President, Professor A. D. MacGillivray, University of Illinois; Secretary, Professor Paul S. Welch, University of Michigan; Treasurer, Mr. Wm. F. Henderson, James Millikin University.

Professor Frank Smith of the University of Illinois, Professor J. E. Ackert of the Kansas State Agricultural College, and Dr. B. H. Ransom of the Bureau of Animal Industry were chosen as the elective members of the Executive Committee for 1920.

Minutes of the last annual meeting were approved as printed.

Adjourned.

Paul S. Welch,
Secretary

CUSTODIAN'S REPORT FOR THE YEARS 1918 AND 1919
SPENCER—TOLLES FUND

Amount reported December 1917		5331.57
June 30, 1918 Dividends	159.93	
Dec. 31, 1918 Dividends	164.73	
June 30, 1919 Dividends	226.24	
Dec. 31, 1919 Dividends	176.46	727.36
		6058.93
less Grant No. 6		100.00
Net amount invested		5958.93

Increase during last two years $627.36.

TOTALS

All contributions		800.27	
All Sales of Transactions		878.38	
All Life memberships		300.00	
All Interest & Dividends		4270.28	
LESS			
All Grants	250.00		
All Dues for Life members	40.00	290.00	5958.93

Life members: (Robert Brown, dec'd.); J. Stanford Brown: Seth Bunker Capp; Harry B. Duncanson; A. H. Elliott; John Hatly.
Contributors of $50 and over; John Aspinwall; Iron City Microscopical Society, Magnus Pflaum; Troy Scientific Society.

(*Signed*) M. PFLAUM,
Custodian.

Philadelphia, Pa., January 10, 1920.

The undersigned having examined the foregoing report certify that we find the amount invested as shown therein $5958.93—correct, as shown by the Pass-Book of the Keystone State B. & L. Association, the same being brought down to the 2nd instant inclusive.

(*Signed*) EDWARD PENNOCK,
EDW. P. DOLBEY,
Auditing Committee.

ANNUAL REPORT OF THE TREASURER OF THE AMERICAN MICROSCOPICAL SOCIETY DECEMBER 22, 1917 TO DECEMBER 21, 1918

RECEIPTS

Balance on hand from audit of 1917			$770.57
Membership dues			548.84
Back volumes	$ 34.84		
For 1918	226.00		
For 1919	286.00		
For 1920	2.00		
Subscriptions		$	370.40
Back volumes	$ 50.00		
Volume 36	95.00		
Volume 37	140.20		
Volume 38	83.20		
Volume 39	2.00		
Initiation fees		$	15.00
Advertisers volume 36			55.00
volume 37			120.00
Sundries			1.09
TOTAL RECEIPTS			$1,880.90

EXPENDITURES

Publishing Transactions		$877.82
Volume 36, number 4	$293.82	
Volume 37, number 1	182.57	
Volume 37, number 2	181.65	
Volume 37, number 3	171.05	
Plates	48.73	
Postage and Express		$59.48
Office Expenses		35.27
Secretary	$23.17	
Treasurer	12.10	
Miscellaneous items		11.85
TOTAL EXPENDITURES		$ 984.42
Balance on hand		896.48
		$1,880.90

Respectfully submitted,

H. J. VAN CLEAVE, *Treasurer.*

REPORT OF OUTGOING TREASURER FOR THE PERIOD OF DEC. 22, 1918 TO FEB. 28, 1919

RECEIPTS

Balance from 1918		$896.48
Membership dues		72.00
Back volumes	$10.00	
For 1919	60.00	
For 1920	2.00	
Subscriptions		47.40
Back volumes	$ 4.00	
Volume 38	41.00	
Volume 39	2.40	
Initiation fees		9.00
Advertisements volume 37		25.00
TOTAL RECEIPTS		$1,049.88

EXPENDITURES

Publication of Transactions Volume 37, number 4		$267.14
Postage and Express		25.26
Office expenses		23.96
Secretary	$ 5.55	
Treasurer	18.41	
Miscellaneous		3.50
TOTAL EXPENDITURES		$ 319.86
Balance transferred to new Treasurer		730.02
		$1,049.88

Respectfully submitted,
H. J. VAN CLEAVE, *Treasurer.*

REPORT OF AUDITING COMMITTEE ON TREASURER'S ACCOUNTS FROM DECEMBER 22, 1917 TO FEBRUARY 28, 1919

This is to certify that we have this day examined the accounts of H. J. Van Cleave, Treasurer, and have checked the vouchers against payments; we find the accounts correct and have verified the bank balance of $730.02 to be transmitted to the incoming treasurer.

HENRY B. WARD
FRANK SMITH
Audit Committee.

ANNUAL REPORT OF THE TREASURER OF THE AMERICAN MICROSCOPICAL SOCIETY
March 1, 1919 to December 24, 1919

RECEIPTS

Balance received from former treasurer	$ 730.02
Dues received from Volume 37 or before	36.00
Dues received from Volume 38	108.00
Dues received from Volume 39	292.10
Dues received from Volume 40	2.00
Initiation fees	66.00
Subscriptions for Volume 37 or before	16.00
Subscriptions for Volume 38	51.80
Subscriptions for Volume 39	34.00
Sales of Transactions, duplicates and back numbers	183.00
Donation from T. B. Magath for aid in publishing his paper	50.00
Advertising for Volumes 38 and 39	55.00
Sundries	4.50
TOTAL	$1,628.42

EXPENDITURES

Printing Transactions, Volume 38, No. 1	$201.03
Printing Transactions, Volume 38, No. 2	265.21
Printing Transactions, Volume 38, No. 3	337.47
Plates for Volume 38, No. 3	76.30
Printing Author's Reprints	20.54
Postage and Express for Secretary	73.92
Postage and Express for Treasurer	22.09
Office expenses of Secretary	126.45
Office expenses of Treasurer	19.70
Sundries	5.67
Balance on hand	480.04
TOTAL CREDITS	$1,628.42

W. F. HENDERSON, *Treasurer*

This is to certify that we have examined the books and vouchers of the Treasurer and find them to be in good condition and to give a correct record of moneys received and expended as indicated.

HENRY B. WARD
H. J. VAN CLEAVE
Auditing Committee

March 24, 1920.

CLASSIFIED ADS

INDEX TO ADVERTISEMENTS

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New Jersey Entomological Co., South Amboy, N. J ..... Third Cover
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Members should bear in mind that our advertisers make possible a larger magazine than could otherwise be issued. We owe them our patronage as well as our thanks.

Nowhere will you find more reliable dealers in materials for the laboratory than those advertised in this magazine.

STATEMENT

of the TRANSACTIONS OF THE AMERICAN MICROSCOPICAL SOCIETY, published quarterly at Menasha, Wis., required by the Act of August 24, 1912.

Name of	*Postoffice Address*
Editor, Paul S. Welch	Ann Arbor, Mich.
Managing Editor, none	
Business Manager, none	
Publisher, George Banta	Menasha, Wis.
Owners,	
President, L. E. Griffin	Pittsburgh, Pa.
Secretary, Paul S. Welch	Ann Arbor, Mich.

Known bondholders, mortgagees, and other security holders, holding one per cent or more of total amount of bonds, mortgages, or other securities:

None.

George Banta, Publisher.

Sworn to and subscribed before me this 29th day of March, 1920.

[SEAL] Gertrude W. Sawyer,
Notary Public.

(My commission expires March 16, 1924.)

ORGANIZED 1878 INCORPORATED 1891

APRIL
1920

VOLUME XXXIX NO. 2

TRANSACTIONS

OF THE

AMERICAN MICROSCOPICAL SOCIETY

PUBLISHED QUARTERLY BY THE
AMERICAN MICROSCOPICAL SOCIETY
MENASHA, WISCONSIN
ANNUAL SUBSCRIPTION $3.00 SINGLE COPY $1.00
DOUBLE NUMBERS $2.00

Attention of members of the

AMERICAN MICROSCOPICAL SOCIETY

is called to the announcement of Grants
from the Spencer-Tolles Research Fund.
(See TRANSACTIONS, July, 1913)
These awards are to stimulate and aid research in
Microscopic work

1. The Grantee must be a member of the Society.
2. Resulting discoveries must first be offered for publication in the Society Transactions.
3. Application should be made to Dr. Henry B. Ward, Chm., Urbana, Ill.

NOTICE TO MEMBERS

The Secretary-Editor desires to secure information concerning sets, partial sets, or odd volumes of the TRANSACTIONS which might be secured for the Society either by gift or purchase. Members knowing of such possibilities are earnestly requested to communicate with the Secretary. The earlier volumes are especially needed.

The Secretary will consider it a favor if members will notify him of non-receipt of numbers of the TRANSACTIONS, and of changes of address.

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PAUL S. WELCH,
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MODERN DARK-FIELD MICROSCOPY AND THE HISTORY OF ITS DEVELOPMENT

BY
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INTRODUCTION

In most work with the microscope the entire field of view is lighted and the objects to be studied appear as colored pictures or as shadows—in extreme cases, as silouhettes—on a white ground. As the field is always light, this has come to be known as Bright-Field Microscopy (Fig. 1).

Fig. 1

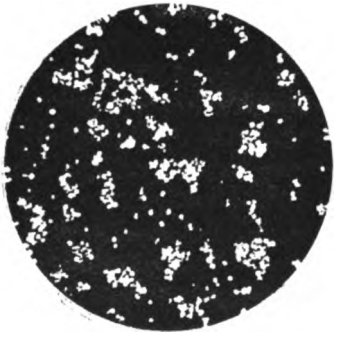

Fig. 2

Bright- and dark-field photo-micrographs of the same objects (starch grains).

In contrast with this is Dark-Field Microscopy in which the field is dark, and the objects appear as if they themselves emitted the light by which they are seen (Fig. 2).

The study of objects in a bright-field probably comprises 95% of all microscopic work, and is almost universally applicable. On the other hand dark-field microscopy has only limited applicability, and yet from the increased visibility given to many objects it is coming to be appreciated more and more.

Definition.—In its comprehensive sense, Dark-Field Microscopy is the study of objects by the light which the objects themselves turn into the microscope, and none of the light from any outside source passes directly into the microscope as with bright-field microscopy.

There are two principal cases: (A) The objects which are truly self-luminous like phosphorescent animals and plants; burning or incandescent objects, and fluorescent objects. (B) The objects which emit no light themselves, but which deflect the light reaching them from some outside source into the microscope.

These two groups are well represented in Astronomy. If one looks into the sky on a cloudless night, the fixed stars show by the light which they themselves emit, but the moon and the planets appear by the light from the sun which they reflect to the earth, the sun itself being wholly invisible at the time. As there is relatively very little light coming from the intervening space between the stars and planets, all appear to be self-luminous objects in a dark field. This reference to the sky at night will serve to bring out two other points with great clearness: (1) The enhanced visibility. Everybody knows that there are as many stars in the sky in the daytime as at night, but they are blotted out, so to speak, by the flood of direct light from the sun in the daytime, while at night when these direct rays are absent and no light comes from the back-ground the stars and the planets show again by the relatively feeble light which they send to the earth.

(2) The other point is that in dark-field microscopy the objects must be scattered, not covering the whole field (Fig. 2). If there were no intervening empty space the whole face of the sky would look bright

It will be seen from this that ordinary sections or other objects so large that they fill the whole field of the microscope cannot be studied advantageously by the dark-field method, for they would make the whole field bright. But for the liquids of the body, blood, lymph, synovial, and serous fluids, fluid from the cavities of the

nervous system, saliva, and all other mucous fluids, and isolated tissue elements where the solid or semi-solid substances are distributed in a liquid, the appearances given by this method are a revelation as was pointed out by Wenham and Edmunds and many others over fifty years ago. No less is the revelation coming from the study of bacteria, protozoa and other micro-organisms in the dark field.

Dark-Field and Ultra-Microscopy

In both of these the objects seem to be self-luminous in a dark field, and no light reaches the eye directly from an outside source, but only as sent to the eye from the objects under observation.

The terms simply represent two steps, and merge into each other.

Dark-Field Microscopy deals with relatively large objects, 0.2μ or more in diameter, that is, those which come within the resolving power of the microscope.

Ultra-Microscopy deals with objects so small that they do not show as objects with details, but one infers their presence by the points of light which they turn into the microscope. This can be made clear by an easily tried-naked-eye observation. Suppose one is in a dark room, and a minute beam of brilliant light like sunlight or arc light is directed into the room. Unless one is in the path of this beam of light it will remain invisible, but if there are vapor or dust particles present they will deflect some of the light toward the eye and will appear as shining points. The character of the particles cannot be made out, but the points of light they reflect indicate their presence. As Tyndall used this method in determining whether a room was free from dust in his experiments in spontaneous generation, the appearance of the shining dust particles is sometimes called the "Tyndall effect."

The two forms are said to merge, because in studying objects like saliva, etc., with the microscope designed especially for dark-field work, some of the objects seen will show details, but some are so small that they show simple as points of light usually in the form of so-called diffraction discs. The larger objects in the saliva come in the province of dark-field microscopy, and the smallest ones, of ultra-microscopy, and in this case the instrument used might with equal propriety be called a dark-field or an ultra-microscope.

The great purpose of the dark-field microscope is to render minute objects or details of large objects plainer or actually visible

from the advantages offered by the contrast given between the brightly lighted objects and the dark background. For example, with the homogeneous immersion objective the study of fresh blood with the ordinary bright-field method enables one to see the red corpuscles with satisfaction, but the leucocytes are not easily found and the blood-dust (chylomicrons) and the fibrin filaments are not seen at all or very faintly. With the same microscope using the dark-field illumination the leucocytes are truly white cells, and the blood-dust is one of the striking features of the preparation, and the fibrin filaments seem like a delicate cobweb.

In this connection, perhaps a few words should be added on the terms Resolution and Visibility. Both came over from the ancient science of astronomy, and are properly used only when restricted as in astronomy.

By resolution is meant the seeing of two things as two, not blended. For example if two stars are close together they are resolved if they appear as two. When the telescope was invented it was found that many stars that appeared single were really two stars close together. If two lines are placed close together they appear as two to the naked eye when close up, but as one moves away the lines seem to fuse and make one. Visibility refers only to the possibility of seeing a thing. In the above examples the twin stars were visible to the naked eye but not resolved into two, and likewise the lines were long visible after they could be seen as two lines. Now the purpose of the ultra-microscope is solely to increase the visibility of small particles without reference to their details of structure. Dark-field microscopy, on the other hand, while it gives greatly increased visibility, also gives resolution of details.

As with bright-field microscopy the resolution of details of structure depends directly upon the numerical aperture (NA) of the objective, and the brightness upon the square of the aperture (NA^2).

Method of Dark-Field Microscopy

In this article the ultramicroscope and the study of self-luminous objects will not be further considered, but the discussion will be limited to objects which must be lighted by some outside source.

There are two principal cases: (1) objects which are lighted from above the stage of the microscope or by so-called direct light (Fig. 3)

and, (2) objects which are lighted from below the stage, or by transmitted light (Fig. 4).

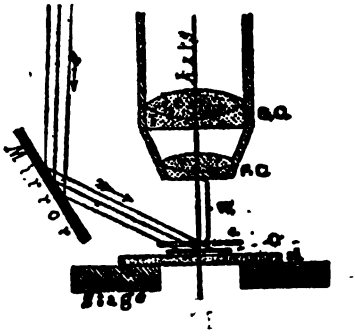

Fig. 3. Light from above the stage. (From *The Microscope*)

In both cases the light from the source is at such an angle that none of it can enter the objective directly but only as it is deflected or "radiated" by the objects in the microscopic field.

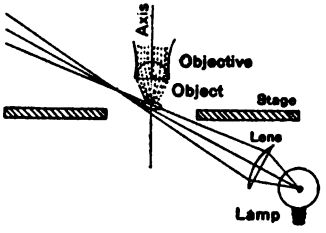

Fig. 4. Light from below the stage.

When the light upon the object is from above the stage the background must be non-reflecting. If the background were white there would be a kind of bright-field, not dark-field microscopy.

The black-background is secured either by placing the object directly upon some black velvet or other non-reflecting surface, or on a glass slide which in turn is placed upon black velvet, etc., or on a dark well. The simplest way to produce a dark-well is to turn the condenser aside and place a piece of black velvet over the foot of the microscope. Or the condenser can be lowered well and the velvet put over the top of the condenser.

Diffuse daylight from a window, or more satisfactorily, artificial light directed by a mirror or lens (bull's eye), is directed obliquely down upon the preparation (Fig. 3). Exactly the same preparation will answer for light from below the stage. In this case the condenser is turned out of the way, and some black-velvet put over the foot of the microscope to cut out stray light.

For a good naked eye demonstration showing the increased visibility due to the dark-field, some cotton may be placed on a piece of black velvet, and a similar tuft of cotton on a white card.

For the special methods of lighting microscopic objects from above the stage, see in the historical summary at the end of this paper.

Dark-Field Microscopy by Transmitted Light.—To make objects appear self-luminous in a dark field when illuminated by beams of light from below the stage, two things are necessary:

(1) The objects must be able to deflect in some way the light impinging upon them into the microscope.

(2) None of the light from the source must be allowed to pass directly into the microscope. These conditions are met when (a) the objects to be studied are of different refractive index from the medium in which they are mounted, and (b) when the transmitted light thrown upon the object is at such an angle that it falls wholly outside the aperture of the objective (Fig. 4-7).

The objects deflect the light into the microscope

(1) By Reflection
(2) By Refraction
(3) By Diffraction

Any one of these will suffice, but any two or all of the ways may be combined in any given case.

For low powers where the aperture of the microscope objective is relatively small it is comparatively easy to make the transmitted beam of so great an angle that none of it can pass directly into the microscope. A simple experiment will show this: A 16 mm. or lower objective is used, the substage condenser is turned aside and on the stage is placed a clean slide with a little starch, flour, or other white powder dusted upon it. If now the mirror is turned to throw the light directly up into the microscope the field will be bright and the objects relatively dark, but if the mirror is turned at an angle suf-

ficient to throw the whole beam at a greater angle than the aperture of the objective will receive, the field will become dark and the starch or flour grains will stand out as if shining by their own light. If some black velvet is placed on the foot of the microscope so no light can be reflected upward into the microscope from the foot or the table, the field will be darker. This experiment succeeds by either natural or artificial light. If some water containing paramecium and other micro-organisms is put on the slide and put under the microscope, the organisms will appear bright and seem to be swimming in black ink.

It is readily seen that with the method just discussed the light is all from one side (Fig. 4). To light the objects from all sides, that is, with a ring of light, the simplest method, and the method utilized in all modern dark-field microscopy, is to use a hollow cone of light, the rays in the shell of light all being at so great an angle with the optic axis of the objective that none of them can enter the microscope directly (Fig. 4-7).

With Refracting Condensers. With the condensers of the achromatic or chromatic type used for bright-field microscopy a solid cone of rays is used. To get the dark-field effect the objects to be studied must be lighted only by the rays at so great an angle that they cannot enter the objective directly. This requires that the condenser shall have a considerably greater aperture than the objective. The ordinary method of making the hollow cone is to insert a dark stop—central stop—to block or shut off the central part of the solid cone of light. The object is then illuminated with a ring of light of an aperture greater than that of the objective (Fig. 6). Some of this light is turned by the objects into the microscope. As only a relatively small amount of the light is deflected by the objects into the microscope, it is evident that there must be a great deal of light to start with or there will not be enough passing from the object to the microscope to make it properly visible. The question also naturally arises how one is to determine the size of the central stop to be used with any given condenser and objective.

This is easily determined as follows: The field is lighted well as for ordinary bright-field observation and some object is got in focus. Then the object is removed and the iris diaphragm of the condenser opened to the fullest extent. If one then removes the ocular and looks down the tube of the microscope and slowly closes

the iris, when the full aperture of the objective is reached, that is, when the back lens of the objective is just filled with light, the opening in the iris represents the size of the central stop to use to cut out all the light which would pass into the microscope from the condenser; all the ring of light outside of this is of too great an angle for the aperture of the objective. One can measure the size of the opening in the iris with dividers and then prepare a central stop diaphragm.

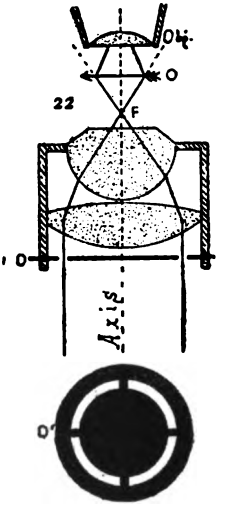

Fig. 5. Ordinary condenser with sectional and face views of the central stop (D). (From *The Microscope*)

A visiting card is good for this. It should be blackened with India ink. To be on the safe side it is wise to make the central stop a little greater in diameter than the iris opening (Fig. 5).

If now the microscope is lighted as brilliantly as possible, and then the iris opened to its full extent and the blackened central stop is put in the ring under the condenser, and a slide used with starch or flour on it, the flour or starch particles will be lighted with the ring of light, and they will deflect enough into the objective to make the objects appear bright as if shining by their own light, the background remaining dark. If the field looks gray or light instead of black it is because the central stop is too small or not centered or the particles used for objects are too numerous, not leaving enough blank space.

One can determine what is at fault thus: The ocular is removed. If the central stop is too small the back lens of the objective will show a ring of light around the outside. If the central stop is not centered there will be a meniscus of light on one side. If the objects are too numerous the whole field will be bright. To verify these statements one can use a specimen with flour or starch all over the slide. It will look dazzlingly light, with the ocular in place and the back-lens will be very bright when the ocular is removed.

For the meniscus of light when the central stop is decentered, purposely pull the ring holding the stop slightly to one side and the meniscus will appear in the back lens. To show the ring of light due to a too small size of the stop, the easiest way is to use a higher objective, say one of 3 or 4 mm. in place of the 16 mm. objective. While it is necessary to eliminate all the light which could enter the objective directly, the thicker the ring of light which remains to illuminate the objects the more brilliantly self-luminous will they appear, therefore one uses only the stop necessary for a given objective. If one makes central stops for the different objectives as described above it will be greatly emphasized that the objectives differ in aperture, in general the higher the power the greater the aperture, and consequently the larger must be the central stop, and the thinner the ring of light left to illuminate the object. As one needs more light for high powers instead of less than for low powers, the deficiency of light caused by the large central stop must be made good by using a more brilliant source of light for the high powers.

Reflecting Condensers. As was first pointed out by Wenham, 1850-1856, refracting condensers are not so well adapted for obtaining the best ring of light for dark-field work as a reflecting condenser, on account of the difficulty in getting rid of the spherical and chromatic aberration in the refracted bundles of such great aperture. He first (1850) used a silvered paraboloid and later (1856) one of solid glass as is now used. Within the last 10-15 years there has also been worked out reflecting condensers on the cardioid principle. The purpose of all forms is to give a ring of light which shall be of great aperture, and be as free as possible from chromatic and spherical aberration, and hence will form a sharp focus of the hollow cone upon the level where the objects are situated.

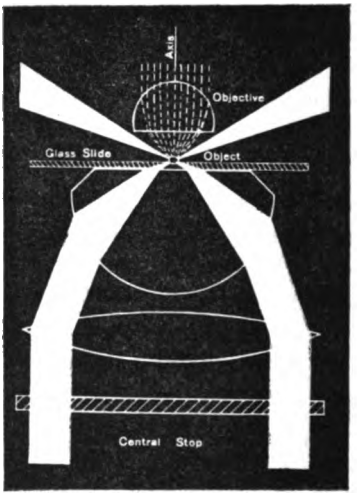

Fig. 6. Bright-field condenser with central stop to give dark-field illumination.

This is a sectional view showing the hollow cone of light focusing on the object and then continuing wholly outside the aperture of the objective.

The light deflected by the object into the objective is represented by broken lines.

The glass slide is in homogeneous contact with the top of the condenser, and the medium beyond the object is represented as homogeneous with glass.

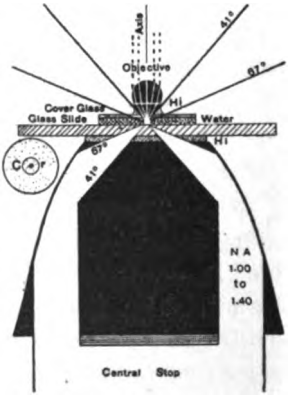

Fig. 7. Paraboloid condenser for dark-field illumination.

Axis—The principal optic axis of the microscope.

Central Stop—The opaque stop to cut out all light that would be at an aperture less than 1.00 NA.

Cover Glass—The cover for the object. For dry objectives it must conform to the objective, and with homogeneous objectives it must be less than their working distance in thickness.

C r—Face view of the top of the paraboloid showing the centering ring, the spot of white ink in the middle and the grains of starch for centering and focusing high powers.

Glass Slide—The slip of glass on which the object is mounted. It is connected with the top of the paraboloid by homogeneous liquid, and must be of a thickness to permit the focusing of the hollow cone of light upon the object.

Hi, Hi—Homogeneous liquid between the cover-glass and the objective and between the top of the condenser and the slide.

NA 1.00 to 1.40—The numerical aperture of the hollow cone of light focused on the object by the paraboloid. As indicated on the left this is represented by a glass angle of 41 to 67 degrees.

41° 67°—The limits of the angle of the rays in glass. Objective—The front lens of the objective. The light rays deflected by the object are indicated by white lines below and through the lens, then by broken, black lines above the front lens of the objective. Water—The mounting medium for the objects.

In this diagram the course of the rays from the paraboloid are indicated as if the objects were mounted in homogeneous liquid and that the rays passed beyond the focus into a medium homogeneous with glass.

TABLE SHOWING THE MAXIMUM ANGLE IN GLASS, AND THE CORRESPONDING NUMERICAL APERTURE OF THE LIGHT WHICH CAN PASS INTO MEDIA OF DIFFERENT REFRACTIVE INDEX ABOVE THE CONDENSER (FIG. 8-11)

	Angle in Glass	Numerical Aperture	Index of Refraction
1. Air over the condenser	41°	1.00	1.00
2. Water	61°	1.33	1.33
3. Glycerin	75° 15′	1.47	1.47
4. Homogeneous liquid	90°	1.52	1.52

In the reflecting as in the refracting condensers the central part of the light beam from the source is blocked out by a central stop and only a ring of light enters the condenser.

Immersion connection of condenser and glass slide bearing the specimen.—While the purpose of the reflecting condenser is to produce a very oblique beam of light for illuminating the objects, it is seen at once that the laws of refraction will prevent the light from passing from the condenser to the object unless the glass slide bearing the object is in immersion contact with the top of the condenser.

That is, for air (index 1.00) above the condenser, the rays in glass at 41°, NA 1.00 and less can pass from the condenser into the air and expand into a hemisphere of light in it (Fig. 8). Rays above 41° are totally reflected back into the condenser.

For water (index 1.33) above the condenser, rays in the glass at 61°, NA 1.33, and less can pass into the overlying water and make a complete hemisphere of light in it (Fig. 9). Rays above 61° are totally reflected back into the condenser.

For glycerin (index 1.47) above the condenser, rays in the glass at 75° 15', NA 1.47 and less can pass from the glass into the overlying glycerin and form a hemisphere of light in it (Fig. 10). All rays at a greater angle are reflected back into the condenser.

For homogeneous liquid (index 1.52) over the condenser, there is no limit to the angle of light that can pass from the condenser to it (Fig. 11).

Immersion Liquid between Condenser and Glass Slide. While water or glycerin answers fairly well it is recommended that homogeneous liquid be used in all cases. At first glance this would seem unnecessary for, as just stated the aperture of the light is limited by the medium of least refractive index between the condenser and the object. Thus objects mounted in watery fluids, and especially those mounted in air would seem to have the illuminating ray that could reach them limited by an aperture of 1.33 in one case and of 1.00 in the other (glass angles of 61° and 41°). This would be true if the objects were suspended in the water or in the air, but many of the particles are not suspended but rest on the glass slide, that is are in so-called *optical contact* with the slide. This being true, the angle of the light which can pass from the condenser to them depends upon their own refractive index, and not upon that of the mounting medium (air or water). This explains also why objects not in optical contact with the slide are rendered more visible by the homogeneous immersion contact of slide and condenser for the scattered light from the particles in optical contact helps to light up particles not in contact.

Another consideration also favors the use of the homogeneous immersion contact of slide and condenser, even for objects mounted in air. Physicists have found (see Wood) that beyond the critical angle, while all light is turned back into the denser medium, it does nevertheless pass one or more wave lengths into the rarer medium to

find, so to speak, an easier place to turn around in. If now any object is near enough the slide to fall into this turning distance of the totally reflected light it may be said to be in optical contact, and the light which meets it will pass into it instead of being totally reflected.

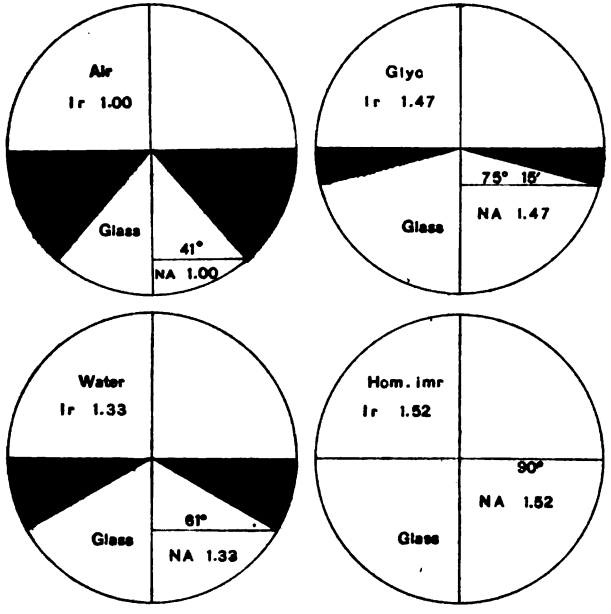

Fig. 8, 9, 10, 11. Diagrams showing the angle and numerical aperture of the light in glass to fill the entire hemisphere above, with overlying media of air, water, glycerin, or homogeneous immersion liquid.

As shown by the diagrams, the NA of the light in each case must equal the index of refraction (Ir) of the overlying medium to fill the overlying hemisphere with light. If the light is at a greater than the critical angle it is reflected back into the condenser. Such light is represented by black in 8, 9, 10. With homogeneous liquid (Hom. imr) above the condenser there is no critical angle.

It should be said in passing that the medium of least refractive index in the path of the light beam from the condenser determines the critical angle at which the light is wholly reflected, and hence determines the maximum angle of the illuminating pencil that can light the object, but this does not apply if the object is in optical contact with the glass (see below).

One can make a very convincing experiment to show the importance of remembering that some of the objects are in optical contact with the glass slide and hence may utilize light which could not pass

into the surrounding medium. If the upper face of the dark-field condenser is cleaned as perfectly as possible, and then lighted well, one can see no light emerging from the top except where the centering ring is situated or where there are some accidental scratches. If one dusts some starch, flour or other white powder on the clean surface, the particles which make optical contact with the glass will glow as if self-luminous. In case one wishes further evidence, the end of the condenser should be carefully cleaned, and a glass slide of the proper thickness connected with it by means of homogeneous liquid, then some flour or starch can be dusted on the slide and it will glow as did the particles on the top of the condenser. These demonstrations show well with the naked eye and with objectives up to 8 mm. (Fig. 7, Cr.)

Aperture of the Ring of Light in the Condenser. As the angle of the light illuminating the objects must be greater than can enter the objective employed it follows that the central part of the illuminating beam must be blocked out up to or beyond the aperture of the objective to be used. The greatest aperture rays possibly attainable depends upon the opticians ability to so design and construct the condenser that it will bring the remaining shell or ring of light to a focus. For those designed to be used with all powers, the aperture of this ring of light usually falls between 1.00 NA and 1.40 NA. As water and homogeneous immersion objectives have a numerical aperture greater than 1.00 NA. it follows that they could not be used for dark-field observation with their full aperture, because much of the light from the condenser could enter the objective, giving rise to a bright or at least a gray field.

Reducing diaphragms for high apertured objectives. As the lower limit in aperture of dark-field condensers is 1.00 NA, and sometimes even lower, it follows that a condenser for use with all objectives requires that none of them have an aperture over 1.00 NA. As all modern immersion objectives have an aperture greater than 1.00 NA, this aperture must be reduced by inserting a diaphragm in the objective.

The general law that the resolution varies directly with the aperture, and the brilliancy as the square of the aperture, holds with dark-field as with bright-field microscopy. In order to determine by actual experiment with various dark-field condensers the best aperture of the diaphragm to select, the writer requested, the Bausch & Lomb Optical Company and the Spencer Lens Company to supply

reducing diaphragms for their fluorite, homogeneous immersion objectives ranging from 0.50 NA. to 0.95 NA. As measured by me these diaphragms ranged from slightly above 0.50 NA, to 0.97 NA. These varying apertures were tested on each condenser, using the same light and as nearly as possible identical preparations (i.e., fresh blood mounted on slides of the proper thickness). It seemed to the

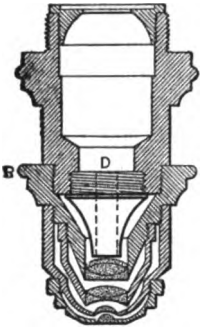

Fig. 12. Large aperture objective with diaphragm to reduce the aperture to less than 1.00 NA. (From Chamot)

D Funnel-shaped reducing diaphragm in the interior of the objective above the back lens.

writer that the law of aperture as stated above held rigidly. The question then is, which aperture shall be chosen if but one diaphragm is available? It seemed to the writer that the one of 0.80 NA should be chosen, at least for these fluorite objectives. If three are to be had the range should be 0.70, 0.80 and 0.90. The reason why one over 0.90 is not recommended is because some examples of the best of the dark-field condensers tested, seemed to have their lower limit somewhat below 1.00 NA, and hence the field could not be made completely dark with the diaphragm of 0.97 NA. With others, however, the field was as dark with this large aperture as with the lower apertured diaphragms.

A considerable range of reducing diaphragms for the homogeneous immersion objectives is recommended because all experience brings home to the worker with the microscope the conviction that some structures show better with the lower apertures and some with higher ones, and it is believed from considerable experience that the same fundamental principles hold in dark-field as in bright-field microscopy.

Lighting for Dark-Field Microscopy

As is almost self-evident, only a very small amount of the light passing through the condenser to the objects is deflected by the objects into the microscope, consequently the source of light must be of great brilliancy or there will not be enough to give sufficient light to render the minute details of the objects visible, when high powers are used. This visibility of minute details involves three things: (1) The aperture of the objectives; (2) The aperture of the illuminating pencil; (3) The intensity of the light.

The most powerful light is full sunlight. Following this is the direct current arc, the alternating current arc and then the glowing filament of the gas-filled or Mazda lamps.

The reflecting condensers are designed for parallel beams consequently the direct sunlight can be reflected into the condenser with the plane mirror of the microscope. If the arc lamp, a Mazda lamp, or any other artificial source is used a parallelizing system must be employed. The simplest and one of the most efficient is a plano-convex lens of about 60 to 80 mm. focus with the plane side next the light and the convex side toward the microscope mirror (Fig. 14) i.e., in position of least aberration. This is placed at about its principal focal distance from the source whether that be arc lamp, Mazda lamp, or any other source and the issuing beam will be of approximately parallel rays. These can then be reflected up into the dark-field condenser with the plane mirror.

Lamps for Dark-Field Microscopy

Up to the present the small arc lamp (Fig. 13), using 4 to 6 amperes is practically the only one considered really satisfactory. There is no question of the excellence of the direct current arc. The alternating current arc has two equally bright craters which renders its use somewhat more difficult.

For most of the work in biology the arc gives more light than is comfortable to the eyes; but a still greater objection is that with the burning away of the carbons the source of light is constantly shifting its position, and hence the quality of the light varies from minute to minute. A third difficulty for hand-feed lamps is that one must stop observation frequently to adjust the carbons.

In spite of all these difficulties, however, the arc lamp is indispensable if one desires to attack all the problems for which the dark-field microscope is available.

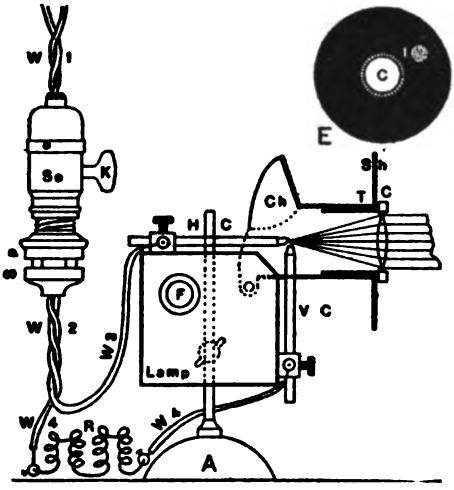

Fig. 13. Small arc lamp for dark-field illumination (From *Optic Projection*) This figure is to show the wiring necessary and the arrangement of the arc and lens to give a parallel beam.

A—Heavy base of the lamp support. By means of a clamp the lamp can be fixed at any desired vertical height. HC and VC, the horizontal and vertical carbons. The HC must be made positive. F, the wheels by which the carbons are fed.

TC—The tube containing the condenser. The condenser in the inner tube can be moved back and forth to get a parallel beam. Sh, black shield, see E.

E—Black shield at the end of the lamp tube (Sh). It serves to screen the eyes and to show when the spot of light is thrown back by the mirror into the parallelizing lens.

W1, W2, W3, W4—The wires of the circuit passing from supply to the upper carbon (HC) and from the lower carbon (VC) to the rheostat, and from the rheostat back to the supply in W1. Never try to use an arc lamp without inserting a rheostat in the circuit. As shown, it forms a part of one wire. It makes no difference whether it is in the wire going to the upper or to the lower carbon, but it must be in one of them.

6-Volt Headlight Lamp.—Next to the arc lamp in excellence for dark-field work is the 6-volt gas-filled headlight lamp (Fig. 14). The reason of this excellence is that the filament giving the light is in a very close and small spiral not much larger than the crater of the small arc lamp, and hence approximates a point source of light.

The brilliancy is also very great as the filament is at about 2800° absolute. The two sizes that have been found most useful by the writer are the bulbs of 72 watts and those of 108 watts. For the bulb of 108 watts a mogul socket is essential; for the 72 watt bulb the ordinary socket is used.

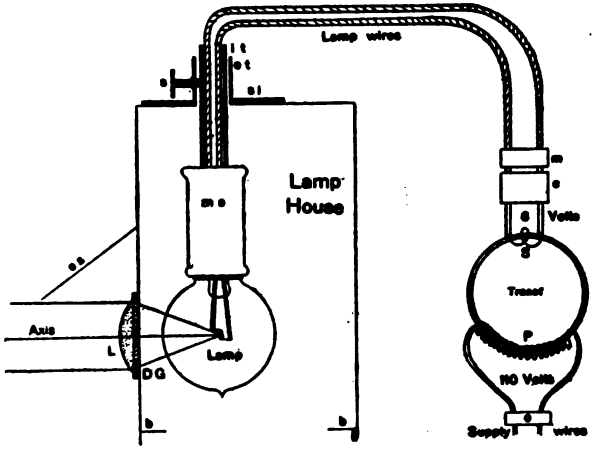

Fig. 14. Diagram of headlight lamp and transformer for dark-field illumination (About one-sixth natural size).

Axis—Axis of the parallel beam from the lens (L).

Lamp—The 6-volt, 108 watt headlight lamp with its very small, close filament centered to the axis of the lens. It is in a mogul socket (ms) and can be centered vertically and horizontally by the inner and outer tubes and set screw (it, ot, s), and the brass slide (sl).

Lamp House—The metal container for the lamp. (b b) Baffle plates near the bottom to help avoid stray light. At the left over the lens (L) is the sloping eye shade. L D G—Parallelizing lens cemented to polished daylight glass.

Lamp wires—The large wires from the transformer (Transf.) to the lamp (Double heater wires are good).

m c—Mistakeless connection between the lamp wires and the transformer (Transf). This is a Manhattan stage connector, and is different from anything else in the laboratory and therefore the lamp can never be connected with a 110 volt circuit and burn out the lamp. Of course any other wholly different connection would answer just as well.

Transf.—Diagram of a step-down transformer. As there are 18 coils around the soft iron ring on the Primary (P) or 110 volt side, and but one coil around the Secondary (S) side, the voltage is stepped down 18 times, or from 110 to 6 volts. In an actual transformer the coils would be far more numerous, but in this proportion. If the transformer were connected wrongly, i.e., with the lamp wires connected with

the primary (P) side, and the 110 volt supply with the secondary (S) side, it would then be a step-up transformer, and raise the 110 volts 18 times—with disastrous results. C, separable connection for the 110 volt supply wires.

The only difficulty with these lamps is that as they are for a 6 volt circuit it is necessary to use a step-down transformer if one has an alternating current with a voltage of 110 or of 220, as is usual.

If one has a direct current of 110 or 220 voltage, then it is necessary to use a storage battery, in general like those used for the lighting and ignition systems of automobiles. As a transformer uses up but a very small amount of energy it will be readily seen that in stepping down the voltage the amperage is correspondingly raised from the general law that the wattage is the product of the voltage into the amperage, and knowing any two the third may readily be found.

For example with the 72 watt lamp, if the voltage is 6 the amperage must be 72/6 or 12 amperes. With the 108 watt bulb the amperage must be $108/6 = 18$ amperes.

The heating of the filament is determined by the amperage, and also it must be remembered that the conductor of an electric current must be increased in due proportion for an increased amperage, consequently in the transformer the wires joining the 110 volt line is small because a very small amperage is necessary to give a large wattage; while from the transformer to the lamp the conducting wires must be large, to carry without heating the amperage necessary with the low voltage (6) to give the large wattage (108 or 72).

For the 18 amperes of the 108 watt bulb, the Fire Underwriters specifications call for wire of No. 12 or No. 14 Brown and Sharp Guage, i.e., wire 1.6 to 2 mm. in diameter or a cable composed of smaller wire having the same conductivity. This specification is for continuous service. In wiring the headlight lamp from the transformer, so called *heater cable* is good, provided one uses a double cable, that is the entire cable for each wire. This is easily done by removing the insulation at the ends and twisting the two strands together, then it can be treated as one wire and the two thus treated used to join the lamp to the mistakeless connection (m c, Fig. 14, 15) of the transformer. As the resistance is small in these large conductors the full effect of the current remains to make especially brilliant the glowing lamp filament, and brilliancy is what is needed for this work.

It should be stated that the transformer for this purpose should be substantial and adapted to continuous service. It is known as a "Bell Transformer" as it is connected to ordinary house light systems for ringing door bells. The one used by the writer was obtained from the General Electric Co. in 1920 and costs at present seven dollars. It is marked: Transformer, type N D, Form P Volts 110 6. Capacity 108 KV-A, Cycles 60, Without taps in Primary." (For making the connections, see the explanation of Fig. 14.)

In comparing the two 6 volt lamps for dark-field work, the 72 watt lamp answers well for most purposes, but the 108 watt one approximates more nearly to the small arc lamp and is sufficient for probably 99% of all dark-field observation in biology. For the remaining 1% one could safely depend on sunlight.

Stereopticon and Mazda lamps for dark-field. In absence of the head-light lamps described above, one can get good results by using in the lamp-house (Fig. 14-15), a stereopticon lamp bulb of 100 to 250 watts. These bulbs have the filament arranged in a kind of ball, and hence fairly well concentrated. This filament must be centered with the parallelizing lens as described for the headlight bulbs. For the horizontal position, move the lamp back and forth by the brass slide until the front of the ball filament is in focus on the 10-meter screen. The microscope should then be placed from 15-25 cm. from the lamp-house. The rest of the procedure is exactly as for the headlight lamp.

If one has neither headlight lamp nor stereopticon lamp, still good work can be done in biology by using the Mazda C bulbs where the filament is in the form of a loop or C. This is centered and focused as for the other lamps (Fig. 18). If one has only a lamp similar to Fig. 18, the daylight glass can be removed and the microscope placed close to the lamp. Fairly good results can be obtained with a 100 watt mazda stereopticon or c bulb without a parallelizing lens.

The Spencer Lens Company recommend in addition to the small arc lamp, their small magic lantern (No. 394). This has either a 250 or a 400 watt stereopticon lamp bulb, and for parallelizing system, the two plano-convex lenses common with simple magic lanterns. The projection objective of the magic lantern is removed. This yields good results especially when a piece of clear daylight glass

is placed over the end of the cone left vacant by the removal of the objective.

A real advantage possessed by these different lights is that the lamps are connected directly with the 110 volt circuit, no transformer being required, as with the headlight lamps. But if one is to do much dark-field work the headlight lamps are much to be preferred.

Daylight effects with the headlight or Mazda lamps. For dark-field work as for work with the bright field, daylight effects are of the greatest advantage both for eye comfort and for the clearness with which details can be made out. The daylight effect is readily obtained by using a piece of daylight glass polished on both sides and cemented to the flat face of the parallelizing lens by means of Canada balsam (Fig. 14-15).

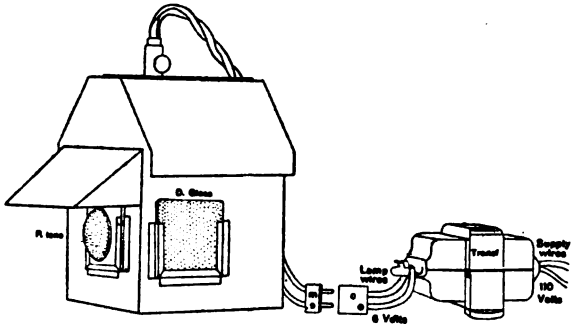

Fig. 15. Headlight lamp in its metal house, and the step-down transformer. (About one-eighth natural size)

D. Glass—The window of daylight glass on the side of the lamp-house to be used for bright-field work. With the glass removed the centering of the lamp is facilitated.

P. lens—Parallelizing lens of about 75 mm. focus. It is cemented to a piece of polished daylight glass.

m c—Mistakeless connection between the lamp wires and the transformer (Transf.). Such a connection prevents joining the lamp with the 110 volt circuit, and thus burning it out. This cannot be connected wrongly.

Transf.—Step-down transformer from 110 to 6 volts.

Lamp-House with centering arrangement. To avoid the non-utilized light, and to place the source of light in the most favorable position, there must be an opaque box to enclose and support the head-light or Mazda lamp. As the filament giving the light must be in the optic axis and practically in the focus of the parallelizing

lens, the lamp or the lens must be sufficiently movable to attain the end. In the lamp-house here figured (Fig. 14, 15) the lens is stationary and the lamp is movable horizontally and vertically, that is, it can be raised and lowered and moved toward and from the lens in the optic axis. For the most perfect centering there should also be arrangements for moving the lamp or the lens from side to side. In the one here shown the parallelizing lens can be shifted slightly to take care of the lateral centering.

Centering the Lamp-filament. As stated above the lamp-filament must be centered, that is, put in the principal optic axis of the parallelizing lens. This is most satisfactorily done by putting the parallelizing lens in position in the lamp-house and measuring the distance from the table to the middle point of the lens. The middle point of lamp filament should be placed at the same height from the table. This is easily accomplished by using the side window of the lamp-house and raising and lowering the lamp by means of the vertical adjustment (Fig. 14-15) until the filament is at the right height to be on the level of the optic axis. Then the lamp is turned until the spiral filament faces the lens. The two limbs of the fork holding the filament then face sidewise. Of course, they would make a shadow if they faced the lens.

To get a parallel beam. The most satisfactory way of doing this is to work at night or in a dark room. Having a white wall or white screen at about 10 meters distant, light the lamp and move it back and forth in the optic axis by means of the top slide (Fig. 14 sl) until the filament of the lamp is in focus on the screen, the filament will then be at about the principal focus of the parallelizing lens, that is, in a position to give approximately parallel light to the microscope. It is well to mark the position on the top of the lamp-house so that if it gets accidentally displaced it can be returned without trouble. It may be said in passing that the lamps are not all exactly alike so that when a new lamp is installed it is necessary to center and focus all over again.

Focusing the crater of the small arc lamp. The makers arrange the carbons and the lens tube so that the crater will be approximately in the optic axis (Fig. 13). Now to get the crater in the focus of the parallelizing lens one can proceed in principle as with the headlight lamp. In the arc lamp, the carbons are fixed and the lens movable. Work at night or in a dark room and with the lighted arc move the

lens back and forth until there is a sharp image of the crater on the 10-meter screen.

Lighting the Microscope. Assuming that the lamp filament or the crater of the arc lamp is centered with the parallelizing lens, one can find the best position for the microscope by holding some thick white paper in the path of the beam and slowly moving out along the beam. Where the spot of light is brightest and most uniform is the best place for the microscope mirror. With the headlight lamps and the arc light this is usually 20-30 cm. from the parallelizing lens.

To get the spot of light to fall on the 45° mirror properly, the center of the mirror must be at the level of the axis of the beam. This can be brought about either by raising the microscope on a block, by inclining the microscope, or by tipping the lamp-house over toward the microscope. If some white paper is put over the mirror one can tell easily when the cylinder of light falls upon it.

To get the light up through the condenser and into the objective it is necessary to so tip the mirror that an image of the source of light is directed back into the parallelizing lens. This image is reflected back from the flat top of the condenser to the mirror. With this arrangement of the mirror the microscope is almost always well lighted, and the mirror will need but a slight adjustment to give the best possible light. This will only be true however, when the source of light is centered to the parallelizing lens and the condenser to the axis of the microscope.

This method of lighting the microscope saves much time and worry. It is effective with the microscope vertical or inclined, with the lamp-house vertical or inclined, and finally it is unnecessary to have the microscope in line with the beam of light. It may be at right angles or at any angle provided the beam of light falls directly on the mirror and the image of the source can be reflected back to the parallelizing lens.

This method of lighting the microscope, so simple and generally applicable, has the one draw-back that the reflected image is rather faint and therefore not easily seen in a light room; at night or in a dark room it is very easily applied. If one is using the headlight lamp and the parallelizing lens is on the outside as shown in Fig. 14-15, one can tell easily when the image is reflected back into the lens from the bright image seemingly considerably nearer the

lamp filament than the blue image of the filament shown in the lens. To see these images one should look obliquely into the lens, that is, along a secondary not along the principal axis. One can also gain help in lighting by turning the mirror till a spot or ring of light appears on the upper end of the condenser. If the slide is in place with the oil for immersion, the spot of light will be bright. One must usually change the mirror slightly after the preparation is in focus to get the best light.

Centering and Focusing the Dark-Field Condenser

As can be seen by Fig. 6-7 the object must be in the focus of the dark-field condenser and this focus must be in the optic axis of the microscope.

The dark-field condenser must have a special mounting with centering screws, which is the common method; or if the microscope has a centering sub-stage arrangement the dark-field condenser need not have a special centering arrangement, but be put in the centering substage fitting. Ordinarily there is no centering arrangement on a microscope and hence the dark-field condenser must have a special centering arrangement of its own. The whole is then placed in the usual bright-field substage condenser ring and raised until it is at the level of the top of the stage. As a guide to centering, there is a circle scratched on the upper surface of the condenser (Fig. 7 c-r). With a low power (16 mm. objective or lower, and x5 ocular) one focuses down on the end of the condenser and if the small circle is not concentric with the circle of the field the centering screws are used with the two hands at the same time and adjusted until the circles are exactly even all around. Unfortunately this is not sufficient for the most satisfactory work, as it is rare that any two objectives will be exactly centered even though screwed into the same opening in the nose-piece, and much less likely to be centered if in different openings. To get the best results the objective to be used and the dark-field illuminator must be centered to each other. To accomplish this the following procedure has been found simple and certain: To start with the dark-field condenser is centered by the low objective as described above, and then with a crow-quill or other very fine pen one puts a very small point of Chinese white or other white ink in the middle of the little centering circle. This is

easily done if an objective of 20 to 40 mm. focus is used for centering the circle on the condenser.

Now for centering the oil immersion or other high power objective the field of which is less than the centering circle, the objective is put in place, but no immersion liquid need be used for the centering. The top of the condenser has dusted upon it some starch or flour or other fine white powder so that in focusing down upon the top of the condenser there will be some shining particles to focus on if the white ink in the center of the circle should happen to be entirely out of the field, which is often the case. When the objective is in focus the centering screws are used to shift the condenser until the minute spot of white ink in the center of the circle is exactly in the middle of the field. In this way any objective may be centered with the condenser, and so far as the centering is concerned, one can be sure of getting the best results of which the condenser is capable.

When the condenser is centered to the high objective, the starch particles and the white ink may be removed with a piece of moist lens paper or a soft cloth.

Focusing the Condenser on the Object Level. This is one of the most essential steps for good dark-field work. If the objects are not in the focus of the condenser they will not be sufficiently lighted so that they can radiate enough light into the microscope to show all their details.

One can proceed as follows, it being assumed that the preparation is mounted on a slide of the proper thickness for the given condenser:—Use a low power, 16 to 50 mm. objective and light the microscope as described in the preceding section. Look into the microscope and focus on a saliva preparation. Move the slide around until there are plenty of epithelial cells in the field and then make slight changes in the mirror until the most brilliant light is obtained. With the screw device for raising and lowering the condenser shift the position up and down slightly until the smallest and most brilliantly lighted point is found. When this is accomplished the condenser is in the optimum focus for that slide and will give the most brilliant light of which it is capable for the source of light used.

Any preparation for examination can have the condenser focused upon it as just described.

For experimental purposes a very satisfactory preparation for focusing the condenser is made as follows: A slide of the right thick-

ness is selected and cleaned and on one face near the middle is painted, with a fine brush, a very thin layer of Chinese white or other white ink. When this is dry, a drop of Canada balsam is put upon it and then a cover-glass. The white particles are very fine and serve admirably to show the focal point of the condenser. Such a slide can be kept as a standard and if the condenser is focused by its aid, it will be in the right position for any preparation mounted upon a slide of the same thickness as the standard. One must always remember, however, that many preparations have an appreciable thickness, and if the slide were of exactly the same thickness as the standard the light might be made more brilliant in a given case by focusing the condenser slightly upward for the higher levels of the preparation. This shows also that the slides selected for preparations should be somewhat under the maximum thickness allowable for the given condenser.

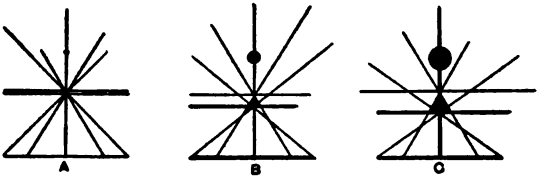

Fig. 16. Face and sectional views of the focus of the hollow cone of light from dark-field condensers

A—Sectional view of an optically perfect dark-field condenser in which the sun is represented as focused nearly to a point. No such condenser exists.

B—Sectional view of a possible condenser focus. It is drawn out somewhat and spreads laterally. The variation in the thickness of slide which might properly be used is shown by the two parallel lines enclosing the elongated focus.

C—Sectional view with a still more elongated focus. The parallel lines show that the variation in thickness of slide permissable is correspondingly increased.

The apparent size of the sun's image is shown on the axis above in each case. It is least sharp in C.

The black line above the letters (A, B, C) represents the top of the condenser.

Thickness of glass-slide to use. Mention has been made of glass slides of the proper thickness. What should this thickness be and how can it be determined are pertinent questions for one who is to get satisfactory results in dark-field work. The thickness of the slide with any given condenser is that which will bring the focus of the condenser—that is the image of the source of illumination—on the upper face of the glass slide where the object is located. Either

on the instrument or in the maker's directions for its use the thickness of slide which should be used with it is given. If such definite information is not available or if a person wishes to determine for himself the proper thickness of slide to use, it may be found out as follows: An arc lamp and a dark room are necessary. The light should preferably be parallelized as shown in Fig. 13. The tube of the microscope is removed, and a piece of uranium glass with plane faces is placed on the stage and connected with the top of the condenser by homogeneous immersion liquid. The uranium glass is strongly fluorescent and shows with great definiteness the exact path of the beams of light from the condenser. One can see exactly where the light comes to a focus above the condenser and then the diverging beams above the condenser. If the condenser were perfect the rays would focus very accurately at a point above the condenser face, Fig. 16 A. This focal point is where the object should be placed and its distance above the condenser face gives the thickness of the slide to use. One can see that with an optically perfect condenser the thickness should be very exact to get the most brilliant image. If the optical system is less perfect as shown in B Fig. 16 the rays do not all cross at one point, but over an appreciable thickness and anywhere within that elongated focus would give a brilliant illumination. In this case the thickness of the slide used could vary the length of this focus.

In Fig. 16 C the focus is much elongated and the slide might vary greatly in thickness and still give a brilliant image. Above the sectional view of the focus in each case is given a face view of the brightest point as described above in getting the focus of the condenser. One can readily see that the more perfect the focus at a point the smaller will be the point of light, and as all the rays are at that point it will be dazzlingly brilliant, while with B and C, where only part of the rays focus at any given level the circle of light will be less brilliant, but correspondingly greater in diameter. The larger circle of light has the advantage of giving a larger illuminated field, but the disadvantage of loss of brilliancy for the most exacting work. It should be mentioned also that as the focus gives an image of the source of light, the size of the source of light will also affect the size of the bright spot seen in looking down on the image. This is finely brought out by using the sun as a source and the arc light or the incandescent light.

One can see also from these figures that if the slide is too thin the objects will be partly in the dark space between the *converging* beams, and if the slide is too thick a part of the objects will be in the dark space between the *diverging* beams. If one sees the face view with a low power in either case there will be a ring of light and a central dark disc. and will look something like the central stop in Fig. 5 D

As the preparations (blood, saliva, etc.) usually studied by the dark-field method have an appreciable thickness it is better to use a slide somewhat thinner than the optimum where the object is almost exactly at the level of the upper surface. If the slide is somewhat thinner the various levels of the preparation can be focused on by the condenser by slightly raising and lowering it as the case demands. For example, if the optimum thickness is 1 mm. it is better to use slides of 0.90 or 0.95 mm. and if the optimum thickness is 1.55 mm. it is better to use one of 1.50 mm. for ordinary preparations.

Thickness of cover-glass and tube-length. These should be strictl in accordance with the construction of the objective. In all mode .. objectives the makers state the tube-length and thickness of cover glass for which unadjustable objectives are corrected. As the dark-field illumination brings out very sharply any defects of correction in the objective, one should select a cover of the thickness, and the length of tube recommended by the maker of the objective. This applies particularly to dry, inadjustable objectives. If the objectives are dry and adjustable then corrections can be made for variations from the standard of cover thickness or tube-length.

If the objective being used is homogeneous immersion, the tube-length must be carefully attended to, but the thickness of the cover-glass is immaterial so long as it is thin enough to fall within the working distance of the objective; of course if it were thicker than that one would not be able to get the objective in focus (Bausch, '90; Gage, '87, 1912).

Practical Application of Dark-Field Microscopy

In the practical application of dark-field microscopy it is self-evident that it can be used successfully only with objects scattered, leaving a certain amount of blank or empty space between the objects. If the object being studied covered the whole field then it would all appear self-luminous and give a continuous bright appearance filling the whole field of the microscope.

In Biology, used in the comprehensive sense applied to it by Huxley, there come naturally the following groups of objects in which it is applicable, and likely to yield much information:—

(A) Unicellular organisms in both the plant and the animal kingdoms. This of course would include the Protozoan Animals, the Bacteria, and other unicellular plants.

(B) In the multicellular animals and plants it includes the natural fluid parts with their cellular and granular contents. In the vertebrates, including man, this would, for example, comprise the blood, and the lymph, with their cellular and granular contents; the tissue fluids, and the fluids in the natural cavities like the pericardial, the pleural and the peritoneal cavities, and the liquid found in the cavities of the central nervous system, the joint cavities and tendon sheaths. It is also of great service in the study of the liquids found in mucous containers, as milk, urine, bile, the saliva, the mucous in the nose, and other organs lined with mucous membrane.

Furthermore it is of help in the study of isolated elements of the body like ciliated cells, etc. In a word it is applicable to the study of all animal and vegetable structures—including the pathologic ones—that are naturally isolated, or that can be artificially separated so that there is sufficient blank space between the structural parts.

Dr. Chamot points out its help in the biological examination of water, in the study of foods, fibers, crystallization phenomena, submicroscopic particles and colloids. He adds further (p. 40): "This method is invaluable for demonstrating the presence of very minute bodies or those whose index of refraction is so very nearly the same as that of the medium in which they occur as to cause them to escape detection when illuminated by transmitted light," i.e., by bright-field microscopy.

Summary of Steps Necessary for Successful Dark-Field Observation

1. A powerful source of light must be available.

2. The dark-field condenser is put in place in the substage, and raised until the top is flush with the upper surface of the stage. The condenser is then accurately centered. If there is an iris diaphragm below the condenser it should be made wide open.

3. A homogeneous immersion objective with reducing diaphragm of about 0.80 N.A. is screwed into one of the openings of the nosepiece of the microscope.

4. Slides and cover-glasses of the proper thickness are made very clean, and put in position for rapid handling.

5. The preparation to be examined—blood, saliva, etc.—is mounted on the slide and covered; the cover-glass is sealed with mineral or castor oil, or with shellac cement.

6. The mounted preparation is held in the hand and one or more drops of homogeneous liquid put on the lower side of the slide opposite the cover-glass. The slide is then put upon the stage so that the homogeneous liquid makes immersion contact with the top of the condenser. The condenser may need to be raised or lowered slightly to make the contact perfect.

7. A drop of homogeneous liquid is put on the cover-glass.

8. The mirror is turned until there is a brilliant point of light in the homogeneous liquid on the cover. The objective is then lowered until it dips into the immersion liquid.

9. The microscope is then focused and the light made as brilliant as desired by turning the mirror.

10. Dark-field microscopy requires more accuracy of manipulation than does ordinary microscopy, but the increased visibility pays for all the trouble. A dimly lighted room is desirable for then the eyes are adjusted for twilight vision and can more easily make out the finest details.

Method of Procedure. As an example of the method to be followed in dark-field work, blood may be used. As pointed out nearly 50 years ago, by Dr. Edmunds, blood with dark-field illumination seems like a new structure, so many things are seen with the greatest distinctness that are wholly invisible or only glimpsed when seen by the bright-field method.

(1) Slides of the correct thickness for the condenser are selected and carefully cleaned.

Cover-glasses are also cleaned and placed where they can be easily grasped.

(2) For obtaining the fresh blood the part to be punctured should be cleaned well with 95% alcohol and then with a sterilized needle or Dr. Morre's Haemospast, the puncture is made. The drop of blood exuding can be quickly touched by a cover-glass, and the cover put on the center of one of the prepared slides. If a small amount adheres to the cover, it will spread out in a very thin layer when placed on the slide. At least one preparation should be made which

appears quite red. In making the preparations one should work rapidly so that the various corpuscles will be in their normal numbers, and the fibrin will be formed only after the preparation is on the slide.

If all the preparations are quite red, after a few minutes, one can be made thinner by pressing firmly on the cover by the ball of the thumb covered with gauze or lens paper. The gauze or paper absorbs the blood which runs out at the edge of the cover. In order to prevent evaporation and to help anchor the cover-glass so that it will not move by the pull of the viscid homogeneous immersion fluid, it is advisable to seal the cover by painting a ring of liquid vaseline (petroleum oil) or castor oil around the edge of the cover. One of the thick preparations should not be sealed, but kept for irrigation with normal salt to show especially the fibrin net-work. When ready to study the blood, put a large drop, or two large drops, of homogeneous liquid on the underside of the slide directly opposite the specimen, and place the slide on the stage of the microscope so that the immersion liquid will come over the face of the condenser. Then a drop of immersion liquid is put on the cover-glass and the objective run down into it. If the lighting is secured as explained above one soon learns to focus on the specimen. In general, the field all looks bright just before the objective gets down to the level for seeing the specimen.

(a) The erythrocytes will appear like dark discs with bright rims owing to the convex borders.

(b) The leucocytes appear as real white corpuscles owing to the granules within them which turn the light into the microscope. If the room is moderately warm—20 C or more—the leucocytes, some of them, will undergo the amoeboid movement, and the picture they present will be a revelation to those who never saw it or only with the bright-field microscope. From the clearness with which everything can be seen the minutest change can be followed, and also the most delicate pseudopod detected. Another striking feature will be noticed in the moving ones, that is, the vigorous Brownian movement of the granules in the part of the leucocyte with the amoeboid movement. In those showing no amoeboid movement there is usually no sign of the Brownian movement of the granules; also if a part of the leucocyte is not undergoing amoeboid movement the particles in it are usually motionless.

(c) The fibrin net-work will be seen like a delicate cob-web between the corpuscles. In different parts of the specimen one can find all the appearances of the fibrin shown in text-books on the blood.

(d) Chylomicrons appear everywhere like bright points in the empty spaces. They are in very active Brownian movement. These chylomicrons will probably be the most unusual part to those studying blood with the dark-field for the first time.*

A very striking view of the fibrin net-work may be obtained by irrigating the thick blood preparation. If a drop of normal salt solution is placed on one edge of the cover-glass and a piece of blotting paper on the other the liquid is drawn through washing out many of the erythrocytes. If the washing out process is watched under the microscope the erythrocytes will be seen gliding over or through the fibrin net-work, or some of them will be anchored at one end and if the current is rapid the corpuscles will be pulled out into pear-shaped forms.

The leucocytes look like big white boulders in the stream, wholly unmoved by the rushing torrent around them.

History

Almost always in human progress two steps must be taken (1) The discovery of the fundamental principles involved, and (2) the development of knowledge in other fields to make the application of the principles possible. Often a long time, sometimes a very long time, intervenes between the first steps and the final rendering of the knowledge a part of the common knowledge of mankind. The development of Dark-Field Microscopy is a good illustration of both the statements made.

*The term *chylomicron* is from two Greek words; χύλος, juice or chyle, μικρόν, any small thing, technically the one-thousandth of a millimeter (μ). I have introduced this word to show the origin of these bodies from the chyle, and to indicate their general average size. Gulliver in 1840–1842, called these minute granules the *molecular base of the chyle* and showed that they were identical in the thoracic duct and in the blood vessels of the same animal. He gave their average size as 1/36,000 to 1/24,000 of an inch. They have been called by others free granules or granulations, elementary particles, etc. In 1896 H. F. Mueller described them as "A never-before observed constituent of the blood" and gave the name of haemoconia, literally, blood-dust. (See Gulliver, Lond. Edin. Phil. Mag. Jan. Feb. 1840; Appendix to Gerber's Anatomy, 1842, and notes in the Works of Hewson, 1846; Mueller, Centralblatt f. allg. Path. u. path. Anatomie, Bd. 7, 1896, pp. 529–539).

The ancient opticians, thousands of years ago, knew well that the principle of contrast was of the highest importance in rendering objects visible; but before this could be applied in microscopy, the microscope itself must be devised. This we see in its simplest form in the convex lenses of Roger Bacon (1266-1267) and in the now rarely used compound form of the Dutch spectacle makers, Jansen and Laprey (1590), composed of a convex objective and a concave ocular (Fig. 17). As a result of the Dutch Compound Microscope, Kepler was led to devise the modern form composed of a convex objective and a convex ocular (1610). But this Keplerian compound microscope has undergone many changes since its first conception and many modifications to render it suitable for giving ability to show the delicate structures in nature with their true appearance. Among these changes may be mentioned the preparation of achromatic lens combinations (Dolland 1757) for telescopes and applied to microscopic objectives between 1820-1830, put on the road to perfection by the introduction of the immersion principle (Hooke 1678, Brewster 1813, Amici 1840-1855) and by the aperture made available by the homogeneous immersion objectives of Tolles 1871-1874, and by the apochromatic objectives of Abbe. Condensers for lighting the object have also played a prominent part from that of Descarts (1637) to those recommended by Brewster (1831) and the homogeneous immersion condensers of Wenham, Tolles (1856 to 1871) and those now regularly made for homogeneous contact with the slide supporting the specimen.

Among the subsidiary discoveries were necessary the arc-light of Davy (1800) and the right-angled arc lamp of Albert T. Thompson (1894) (Fig. 13) and the electric generators now everywhere available. In these last days also the gas filled or Mazda lamps with their close filaments of Tungsten which approximate in brilliancy and compactness of source to the arc lamp and greatly excel it in convenience; and lastly of the production of a glass filter to give the light of the tungsten incandescent lamps true daylight quality, and make microscopic work by this artificial light as comfortable as the light from the northern sky (see Ives 1914, Gage 1915-1916).

The time also between the first appreciation of the dark-field for the study of microscopic objects by Lister (1830), Reade (1838), Wenham (1850), Edmunds (1877), and the appreciation of the microscopical worker in general, came only after the invention of the ultra-

microscope (1903) and the application of the dark-field method to the study and detection of pathologic micro-organisms especially the Spirochaeta pallida (1905). It now promises to give much help in working out the activities and minute details of microscopic structure in animals and plants from the lowest to the highest.

In the earliest stages of microscopic study the objects were seen by the light which they directed toward the microscope, and if over a dark background they appeared with varying degrees of brightness as if self-luminous; but even as early as 1637 (Fig. 17) Descartes microscope had provision for sending the light through the object. In this case much of the light did not reach the object at all, but passed on directly to the microscope. This mode of lighting showed the object more or less as a dark body on a brilliant background.

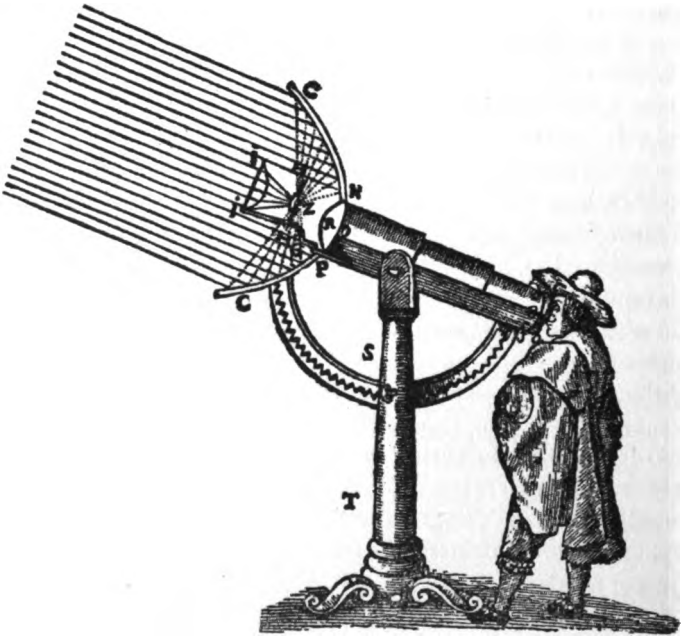

Fig. 17. Descartes Dutch compound microscope with a parabolic reflector and a condensing lens (From Descartes Dioptrique, 1637).

Ocular and Objective. The ocular is a plano-concave lens or amplifier, and the objective (N O P R) is a double convex lens.

Reflector and Condenser. For objects to be lighted from above, there is a parabolic mirror (c c); for those to be lighted from below there is a condensing lens (i i).

These two forms of lighting differed fundamentally in that with the first no light from the source passed into the microscope; but only that from the object, while with the second the light from the source as well as from the object got into the microscope.

The significance of this fundamental difference for the aperture of the objective and for dark-field microscopy were first appreciated by Lister (1830), Wenham (1854), and Gordon (1906), and was practically applied in the manufacture of dark-field apparatus by Zeiss (1904) and Leitz (1905). In a word, it was the appreciation, as stated by Lister (1830) that if the direct light from the source after it had reached the object, were prevented from entering the objective, by blacking the central part of the objective, then only the marginal part of the objective would be functional and that would receive only those rays from the object that were directed to it by the object itself, that is scattered light reflected, refracted, or diffracted, from the object, none of the light from the source getting directly into the microscope. As stated by Wright (p. 217) this is the method of dark-field microscopy by lighting the object with a solid cone of small aperture and, imaging it by hollow beams of large aperture. In practice this method has been discarded for the one by which the object is lighted by beams of light in such a direction with reference to the axis of the objective that none of them can enter the objective directly, and the light going to the microscope comes only from the objects themselves; they will therefore appear self-luminous on a dark background.

The two conditions are (a) where the light is directed upon the object from above and, therefore away from the objective, and (b) where the light is directed upon the object from below, and therefore toward the objective (Fig. 3-4).

If the light is directed upon the object from above and the object is over a non-reflecting background, the object will appear bright in a dark field. Of course, if it is on a light background that will also reflect light into the microscope and both object and background will appear light. It is assumed here that the object or objects cover only a part of the field, leaving plenty of empty space for background.

In striving after a truly non-reflecting background three distinguished men found the same thing, viz., that the only really black thing in nature is a black hole, that is, a space with black walls into which the light cannot enter directly. The dark walls absorb any

stray light, and the empty space gives no reflection. The first of these men devised for his microscopic purposes such a non-reflecting background by means of a small cup or well with the walls painted black. It is known as Lister's black well (1826). The second discoverer was Chevreul (1839), who found in his work on Contrasts that a black space gave the only non-reflecting background. Such a background was used by Marey for making moving pictures to show animal movements. Marey called it Chevreul's black. The third was J. H. Comstock (1901) who found in the study and photography of spider webs that no pigment or fabric was black enough for a background He therefore devised a deep box with the inner walls covered with black velvet and placed it so that the light could not shine into it. Over the mouth of this box the web was placed and lighted at right angles to the opening of the box. The feeble light the webs reflected served well for photography.

These three men then absolutely independently found the same solution to their problem and doubtless many others have found also that Lister's, Chevreul's, and Comstock's black space is the only really black thing in nature.

From the time of Descartes (1637) the means for lighting objects from above the stage have been many. Some of them, like the bull's eye condenser (Fig. 4, lens) and the side reflector send the light only from one side, while with the circular mirror of Descartes (Fig. 17) and the somewhat similar Lieberkuhn reflector (1740) the light is reflected from all sides upon the object. If now the object is on a dark background, it will appear as if self-luminous.

From 1850 to the present two additional means have been devised for lighting from above. The first, following the suggestion of Riddell (1852) aims to make the objective its own condenser, the light being introduced into the side of the objective and reflected down by a small mirror or a prism (H. L. Smith 1865, Tolles 1866). (For a full discussion see W. A. Rogers, Journal of the Royal Micr. Soc., 1880, p. 754-758.)

The other method referred to is that of Prof. Alexander Silverman of the University of Pittsburgh. It consists of a circular electric lamp and reflector which surrounds the objective and shines down upon the object.

Of course all objects lighted from above the stage will give true dark-field effects only when there is a black background, and the objects are scattered, leaving empty space between them.

Dark-Field Microscopy with Substage Illumination. The first specific discussion of the possibility of dark-field microscopy with light from beneath the stage is found in a paper by the Rev. J. B. Reade of Cambridge University and is dated at Peckham, Nov. 1836, and is published as appendix No. 2 in the Micrographia of Goring and Pritchard, 1837. Reade says: p. 229: "To illustrate the two methods (Bright-field and dark-field) by reference to the telescope it may be observed that the discomfort of viewing spots on the sun not unaptly corresponds with the view of microscopic objects on an illuminated field; while the removal of all inconvenient and ineffective light from the field of the microscope corresponds with the clear and quiet view of stars on the dark blue vault of the firmament." He brings out very clearly in his paper that no light from the source shall pass directly into the microscope, only that from the object, and that the object appears "sparkling with exquisite lustre on a jet-black ground."

The first appearance of this method in the general literature of microscopy which was found occurs in John Quekett's Practical Treatise on the Use of the Microscope, 1st ed. 1848, pp. 178-179. He also furnishes a diagram to illustrate the method of lighting something like fig. 4 of the present article, and remarks: "The method consists in illuminating the object by a very powerful light, placed at such an angle with the axis of the microscope that none of the rays can enter it except those which fall directly upon the object, and are so far bent as to pass through it into the compound body," i.e., into the tube of the microscope.

It is referred to in the first edition of W. B. Carpenter's "The Microscope and its Revelations" (1856) as follows:

"Whenever the rays are directed (from below the stage) with such obliquity as not to be received into the object-glass at all, but are sufficiently retained by the object to render it (so to speak) self-luminous, we have what is known as the *black ground illumination;* to which the attention of microscopists generally was first drawn by the Rev. J. B. Reade in the year 1838 (1836-1837) although it had been practised sometime before not only by the author (Dr. Carpenter) but by several other observers."

In addition to the condensing lens of Reade for throwing the very oblique beam of light upon the object, the mirror was used for low powers, and for higher powers, prisms were used especially by Nachet and Shadboldt (1850). It was seen however, that light from only one side might give rise to false appearances.

In the third volume of the Transactions of the Microscopical Society of London, there appeared an epoch-making paper for dark-field microscopy. It is entitled "On the Illumination of Transparent Microscopic Objects on a New Principle." It was read by its author, F. H. Wenham, April 17, 1850. After discussing the prisms of Nachet and pointing out the defect of oblique light from one side only giving rise to false images, he proceeds to show how the defect may be obviated by using two prisms giving light from opposite sides, or, and this is the epoch making part of the paper for dark-field work, by using a truncated parabolic reflector to give a circle of light. A dark stop was present to cut out all but the rays which exceed the aperture of the objective "So that the light which enters the microscope shall be that which radiates only from the object, as if it were self-luminous." The parabolic speculum was truncated so that the light would focus on an object mounted upon the ordinary glass slide.

From this fundamental beginning, illumination by a hollow cone of light by the aid of the truncated parabola, all the advances in dark-ground illumination have proceeded. In 1851, Mr. Shadboldt says: "In order to obviate the objectional shadow (of lighting from one side only) as well as to procure a more brilliant illumination the parabolic condenser was projected by Mr. Wenham, to whom alone belongs the credit of having suggested the use of oblique illumination in *every azimuth*, so as to produce a black field." In this paper Mr. Shadboldt commends the use of a condenser made wholly of glass and depending upon internal reflections to take the place of the metallic parabolic mirror of Wenham. This he named a spheroannular condenser. In considering the obliquity required to have all of the light going to the object of an angle to fall outside the aperture of the objective, it seems to Shadboldt highly desirable that each objective to be used in dark-field work should have its own special condenser. That he understood as perfectly as we the possibility of using a single condenser for all objectives is shown by the following quotation, p. 157, "It is highly desirable that the

condenser should be constructed specially with reference to the aperture of the object-glass with which it is intended to operate; and for a reason to be given immediately, it will be seen that cutting off some of the rays, in order to make a condenser work with objectives of very much larger aperture, although quite practicable and even generally in use with the parabolic condenser, is not nearly so advantageous as the use of a separate condenser for every object-glass . . . of high power at least."

In 1856 Mr. Wenham himself advocates the use of a truncated paraboloid of solid glass with a central stop to cut out all the central rays which would not be internally reflected from the upper surface of the paraboloid. He brings out in the clearest manner possible the need of using immersion contact with the paraboloid to permit the very oblique rays to pass out of the paraboloid into the overlying substance. If the object is in water, then water immersion and when the object is mounted in balsam, he advocates the use of an immersion liquid between the glass slide and the paraboloid of camphine, turpentine or oil of cloves as their refractive index is nearly the same as crown glass and permits the passage of the rays of great aperture to pass on into the slide and the balsam containing the objects. We now use cedar oil or other homogeneous liquid for the same purpose.

In 1877 Dr. James Edmunds presented before the Quekett Microscopical Club a paper on "A New Immersion Paraboloid Illuminator." It consisted of a paraboloid of glass cut off at an exactly calculated distance below the focus, this distance varying in the four lenses which constituted his set, and the plane top being made optically continuous, and as nearly as possible optically homogeneous with the substance of the slide, by means of a cementing fluid of high refractive index, such as anhydrous glycerine, castor oil, copaiba-balsam, oil of cloves, etc. The paraboloid lenses acted on the principle of total internal reflection, and each one was calculated for the thickness of the slide beneath which it was to be used (1/16th in 1/100 inch slides) so as to converge upon the object all of the light entering the base of the paraboloid. Parallel light should be thrown into the base of the paraboloid, and the most splendid effects were obtained by means of direct sunlight. Water immersion objectives of 1/16th and 1/8th inch focus were used. After speaking of some test objects he says, p. 19: "With bacterial fluids, the effect

was equally remarkable. Saliva, blood, etc., viewed by a good dry quarter of about 95° (NA 74), were seen almost as new objects when lighted up by this paraboloid."

As it was recognized from the time of Reade that to gain the dark-field effect the light going to the object must be of an obliquity so great that it could not enter the microscope directly; this involved either a paraboloid or other dark-field illuminator of such great range that it might be used with all objectives, or the suggestion of Shadboldt must be followed that each objective have a paraboloid especially constructed to give it the best possible effect. This question naturally became very insistant when the water immersion objectives of large aperture came into use, and especially when the homogeneous immersion objectives came into common use (1880-1890). It has finally been settled by adopting the first possibility, viz., the use of dark-field illuminators adapted for all objectives, the aperture of the objectives being reduced, where too great, to a point somewhat below 1.00 NA. This makes it possible to utilize a ring of light between 1.00 and 1.52 NA for the dark-field illumination, and this ring of light produced by the sun or the electric light has been found sufficient for practically all dark-field microscopy. It should be stated in passing that the ring of light produced by the dark-field illuminators usually falls between 1.00 NA, and 1.45 NA. Some fall below 1.00 NA and some only go to 1.30 or 1.35. The reducing diaphragms for homogeneous immersion objectives which have come to the writer with objectives have ranged from 0.40 NA to 0.80 NA.

From 1907-1910 papers were written describing and figuring reflecting condensers made on the cardioid principle to take the place of the truncated paraboloid in dark-field work. The effort was made to so figure the component segments of glass that the spherical and chromatic errors would be largely eliminated, and that the entire ring of light could be brought to a more perfect focus than is possible with the truncated paraboloid: that is, to be optically more like A than like B or C in Fig. 16. A simple plate form for use on the top of the stage has also been devised. When this is used the substage condenser is turned out so that the light can pass directly up from the plane mirror to the condenser. This form is not easy to keep accurately centered. From the writer's experience with quite a variety of these dark-field condensers in biological work

the paraboloids have proved the easiest to work with and the most generally satisfactory.

As a final word,—now that the means have been found for fuller microscopic revelations, it behooves biologists to make the most of them; and in the study of the finest details in living things by this dark-field lighting, perhaps a truer conception of structure and action can be gained than by a too exclusive dependence on dead material treated with the endless variety of fixers and stains.

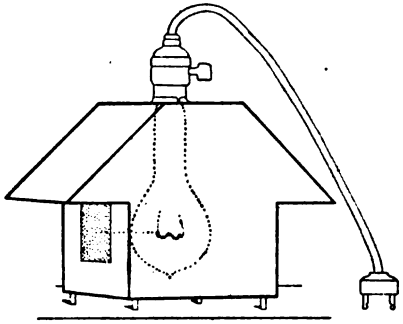

Fig. 18. Chalet microscope lamp for bright-field microscopy (Two-fifteenths natural size).

The lamp has two daylight-glass windows under the overhanging roof. The roof serves to shade the eyes. The source of light is a 100 watt Mazda C lamp bulb, the filament of which is centered with the windows.

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1890. The full utilization of the capacity of the microscope and means for obtaining the same. Proc. Amer. Soc. Microscopists, Vol. XII, 1890, pp. 43-49.

Among other matters Mr. Bausch gives a very thoughtful discussion of the effect of the cover-glass and of tube length.

BORELLUS, PETRUS

1655. De vero Telescopii inventore, cum brevi omnium conspiciliorum historia. Ubi de eorum confectione, ac usu, seu de effectibus agitur, novaque quaedam circa ca proponuntur. Accessit etiam centuria observationum microcospicarum. Authore Petro Borello, regis christianissimi conciliario, et medico ordinario. Hagae-Comitum, ex typographia Adriani Vlacq, MDCLV (1655).

Evidence from those with personal knowledge that telescopes and microscopes were made by the Dutch spectacle makers, Zacharias Jansen, and Hans Laprey, 1590.

CARPENTER, WILLIAM B.

1856. The Microscope and its Revelations. First edition 1856.

An admirable statement of dark-field microscopy is given with the apparatus devised up to that time for effecting it. Showing how greatly dark-field microscopy had been discarded in England one can compare the first and the 6th (1856-1881) editions of this work with the 8th edition, (1901).

CHAMOT, EMILE MONNIN

1915. Elementary Chemical Microscopy. New York, 1915.

This work is recommended not only for the account given of dark-field microscopy and its application, but for the ultra-microscope, the polariscope, the micro-spectroscope and indeed all other chemico-physical apparatus used with the microscope, and their application in chemical and physical investigations.

CHEVREUL, M. E.

1838. De la Loi du Contraste simultané des Couleurs et de l'assortiment des objets colorés considéré d'apres cette loi. Paris, 1839. Work written 1835-1838. Third English edition, 1890. Part of Bohn's Scientific Library.

COMSTOCK, J. H.

1912. The Spider Book. A manual for the study of spiders and their near relatives, the scorpions, pseudoscorpions, whip-scorpions, harvestmen, and other members of the class Arachnida, found in America north of Mexico; with analytical keys for their classification and popular accounts of their habits. New York.

In this book are given pictures of the spider webs photographed against a black space, i.e., a deep box lined with black velvet. See p. 181. The first photographs made in this way were taken in 1901. They were exhibited before the Entomological Society of America at its first meeting, Dec. 28, 1906.

CONRADY, A. E.

1912. Resolution with dark-ground illumination. Jour. Quekett Micr. Club, Vol. 11, (1912) pp. 475-480.

He says: "To get the utmost resolving power with dark-ground illumination, the condenser must have not less than three times the NA of the objective. If the condenser has less than three times the aperture of the objective then the limit of resolution is found by taking ¼ the sum of the apertures of objective and condenser: e.g., if cond. has NA of 1.40, and of obj. 1.00 NA, their sum is 2.40, ¼ of 2.40=0.60 NA; limit in this case."

COX, HON. JACOB D.

1884. Robert B. Tolles and the angular aperture question. Proc. Amer. Soc. Microscopists, Vol. VI, (1884) pp. 5-39.

This very able address, one of the ablest our society ever had the fortune to hear from its president, brings out with absolute clearness and fairness the steps in progress and the role played by Robert B. Tolles in actually making possible the final step, and taking that step, in his homogeneous immersion objectives. That is not all, he published the formula by which the objectives were made. The reading of this address is most strongly recommended to our younger members.

DESCARTES (LAT. CARTESIUS), RENÉ

1637. Oeuvres, Publiées par C. Adam et P. Tannery sous les auspices du ministère de l'instruction publique, Vols. I-XII. Paris, 1902.

The Dioptrique is in Vol. 6 of this edition, and the French and the figures are as in the original of 1637. In Cousin's edition the figures are often considerably modified and the French modernized.

DOLLOND, JOHN

An account of some experiments concerning the different refrangibility of light. Read June 8, 1758. Philos. Trans. Roy Soc. Lond. 1758, pp. 733-743. This is the original paper on achromatic telescopes, etc.

EDMUNDS, JAMES

1877. On a new immersion paraboloid illuminator. Jour. Quekett Micr. Club, Vol. V, (1877) pp. 17–21. Monthly Micr. Jour., Vol. XVIII, 1877, pp. 78–85.

The Paraboloid was made optically continuous and as nearly as possible, optically homogeneous with the slide by the use of anhydrous glycerin, castor oil, copaiba-balsam or oil of cloves. He says that saliva, blood, and bacterial fluids gave remarkable effects, and were almost like new objects when seen with this paraboloid.

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1917. The Microscope, an introduction to microscopic methods and to histology. 12th revised edition, Ithaca. 1917.

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1914. Optic Projection. Principles, installation and use of the magic lantern, the projection microscope, etc. Ithaca, 1914.

GAGE, S. H.

1887. I. Microscopical tube-length and the parts included in it by the various opticians of the world. II. The thickness of cover-glass for which unadjustable objectives are corrected. Proc. Amer. Soc. Microscopists, Vol. IX, 1887, pp. 168-172.

This paper gave the information that has led to greater uniformity.

GAIDUKOV, N.

1910. Dunkelfeldbeleuchtung und Ultramikroskopie in der Biologie und in der Medizin. 5 plates, 81 pages. Jena, 1910.

There is a bibliography of books and papers covering 9 pages (202 titles).

GORDON, J. W.

1907. The top-stop for developing latent power of the microscope. Jour. Roy. Micr. Soc., 1907, pp. 1-13. See also Wright, pp. 216-217.

The plan is to cut out all of the central beam by a stop at the eye point instead of by opaqueing the central part of the objective.

GORING AND PRITCHARD

1837. Micrographia, containing practical essays on reflecting, solar, oxy-hydrogen gas microscopes, micrometers, eye-pieces, etc. 231 p. Many figures in the text, one plate. Whittaker & Co., Ave-Maria-Lane, London, England. 1837. Rev. J. B. Reade on dark-field, pp. 227-231.

HALL, JOHN CHARLES

1856. On an easy method of viewing certain of the Diatomaceae. Quart. Jour. Micr. Sci., Vol. IV, (1856) pp. 205-208.

In this paper Dr. Hall figures natural size, the "spotted lens" of that time, i.e., a very thick, more than hemisphere of glass with the central part opaqued. (See Quekett, 3d. ed., p. 135 where it is said that it is the invention of Thomas Ross.) Hall used this spot lens for oblique light with the ordinary bright field microscopy. He expresses astonishment that this instrument, designed to give dark-field effects, should give bright ones. He did not consider the fact that the aperture of this spot lens was insufficient to throw all the light outside of the aperture of the objective. One would get the same effect if a wide-angled homogeneous immersion were used with a paraboloid, and no reducing diaphragm were put into the objective.

HEIMSTÄDT, OSKAR

1907. Neuerungen an Spiegelkondensoren (Aus der optischen Werkstätte von C. Reichert in Wien). Zeit wiss. Mikr., Bd. XXIV, (1907) pp. 233-242.

HEIMSTÄDT, OSKAR

1908. Spiegelkondensor und Paraboloid. Zeit. wiss. Mikr., Bd. XXV, (1908) pp. 188-195. Erwiderung an Herrn O. Heimstädt, by Siedentopf, pp. 195-199.

Dr. Heimstädt objects to some of Dr. Siedentopf's statements in his paper, "Die Vorgeschichte der Spiegelkondensor." Perhaps the spirit of the polemic will best be brought out by a quotation from Heimstädt, p. 188. "Vol allem beeinträchtigt es den Wert und auch die Neuheit dieser Dunkelfeldbeleuchtung nicht im geringsten, dass dabei längst vergessene Methoden älterer englische Optiker wieder verwendt wurden." In a word, it is well brought out in these papers where the fundamental ideas came from.

IGNATOWSKY, W. V.

1908. Ein neuer Spiegel-kondensor. Zeit. wiss. Mikr., Bd. XXV, (1908) pp. 64-67 with figures of the substage and the plate form. See also Jentzsch, and Siedentopf. Jour. Roy. Micr. Soc., London, 1911, pp. 50-55.

JENTZSCH, DR. FELIX

1911. The reflecting concentric condenser. Physikalische Zeitschrift, Bd. XI, pp. 993-1000. See also Ignatowsky and Siedentopf, Jour. Roy. Micro. Soc., 1911, pp. 50-55.

KEPLER, JOHANNES

1604. Opera Omnia, Vol. II. Ad Vitellionem Paralipomena. (De modo visionis et humorum oculi usu.) 1604, pp. 226-229. 11 figs.

Correct dioptrics of the eye here given, and also the explanation of the effect of convex and concave spectacles.

1611. Dioptrica.—Demonstratio eorum quae visui et visibilibus propter conspicilla non ita pridem inventa accidunt, pp. 519-567. 35 figs., 1611.
The amplifier, real images, and erect images. The Keplerian microscope (Modern microscope.)

LISTER, JOSEPH JACKSON
1830. On some properties in achromatic object-glasses applicable to the improvement of the microscope. Philos. Trans. Royal Society London, Vol. 120 (1830) pp. 187-200.
On p. 191 he discusses the effect of a "Stop behind the object-glass" (retro-objective stop) by which only the outer zone of the objective is used, the central zone being stopped out. See Wenham, 1854.

MAREY, ETIENNE JULES
1901. The history of Chronophotography. Annual Report of the Smithsonian Institution for 1901, pp. 317-340.
On p. 320 Marey refers to Chevreul's method of obtaining perfect blackness.

MAYALL, JOHN, JUN.
1885. Cantor Lectures on the Microscope. Lectures delivered before the Royal Society of Arts, Nov. Dec. 1885.
On pp. 95-96 are given the facts regarding the working out and production of homogeneous immersion objectives. Tolles is given due credit.

MOORE, DR. V. A.
1897. The Hemospast, a new and convenient instrument for drawing blood for microscopic examination. Trans. Amer. Micr. Soc., Vol. XIX (1897) pp. 186-188.
After using this "spring needle lancet" individually and with large classes for many years I quite agree with Dr. Moore when he remarked to me the other day, "It is the most humane instrument I have ever seen for drawing blood." I would like to add to this: And one of the most efficient.

QUEKETT, JOHN
1848. A practical treatise on the use of the microscope including the different methods of preparing and examining animal, vegetable and mineral structures.
First edition, 1848. Reade's method given and illustrated pp. 178-179; Second edition, 1852, Reade's method illustrated pp. 194-195. Third edition, 1855, Reade's method, the method of Wenham, Spot-Lens method of Thomas Ross, the methods of Schadboldt and Nobert are all given.

READE, REV. J. B.
1837. On a new method of illuminating microscopic objects, pp. 227-231 of Goring and Pritchard's Micrographia, which see. (1837).

ROGERS, WM. A.
1880. On Tolles' interior illuminator for opaque objects. (With note by R. B. Tolles). Jour. Roy. Micr. Soc. London., Vol. III (1880) pp. 754-758.
In this paper Rogers gives the history of the devices for making the objective its own condenser by introducing light into its side and reflecting the light down upon the object.

SHADBOLT, GEORGE

1851. Observations upon oblique illumination; with a description of the author's Sphaero-annular condenser. Trans. of the Micr. Soc. of London. Vol. III, pp. 132, 154.

This paper was read in 1851. As this condenser is like the glass paraboloids now used for dark-field work, they are often called the Wenham-Shadbolt paraboloids. Shadboldt discusses prisms in this volume.

SIEDENTOPF, H.

1907. Paraboloid-Kondensor, eine neue Methode für Dunkelfeldbeleuchtung zur Sichtbarmachung und zur Moment-Mikrophotographie lebender Bakterien, etc. Zeit. wiss. Mikr., Bd. XXIV, (1907) pp. 104-108.

1907. Die Vorgeschichte der Spiegelkondensoren. Zeit. wiss. Mikr., Vol. XXIV (1907) pp. 382-395. 16 figures are given of early forms.

1908. Mikroskopische Beobachtungen bein Dunkelfeldbeleuchtung. (Mitteilung aus der optischen Werkstätte von C. Zeiss, Jena) Zeit. wiss. Mikr. Bd. XXV (1908), pp. 273-282. Two plates of photomicrographs of the rays above the different condensers. See also under Heimstädt.

1910. Cardioid-Condenser. Jour. Roy. Micr. Soc. Lond., 1910, pp. 515. See also Ignatowsky, and Jentzsch, Jour. Roy. Micr. Soc. Lond., 1911, pp. 50-55, where will be found a statement concerning the historical relation of these different condensers.

STEPHENSON, J. W.

1879. A catoptric, immersion illuminator. Jour. Roy. Micr. Soc. Lond., Vol. II (1879) pp. 36-37.

This condenser does not depend on internal reflection, but by a silvered surface around the central part. According to Siedentopf this is the condenser copied by Reichert; and according to Heimstädt Wenham's truncated paraboloid was copied by Zeiss (See under Heimstädt).

WENHAM, F. H.

1850. On the illumination of transparent microscopic objects on a new principle. Trans. Micr. Soc. Lond., Vol. III. (1850) pp. 83-90.

This is the paper by Wenham in which dark-field illumination is produced by a hollow silvered parabolic speculum.

1854. On the theory of the illumination of objects under the microscope with relation to the aperture of the object-glass, and properties of light; with practical methods for special differences of texture and colour. Quart. Jour. Micr. Sci. Vol. II (1854) pp. 145-158.

In this paper Wenham refers to the method of Lister (1830) for darkening the central zone of the objective so that no light can enter the outer zone, unless, as Wenham says, it is "*radiated*" from the object (See his fig. 1, and pp. 149-150 of the article). On p. 153, in the reference to the effect that his paper of 1850 had had in the microscopical world he says, "As proof of the utility and correctness of my theory, I have only to mention the many applications of it that have since that time (between 1850 and 1854) come into general use, in the way of adapting central stops to the achromatic condenser, single (i.e., "spot lenses") and compound lenses, etc."

1856. On a method of illuminating opaque objects under the highest powers of the microscope. Trans. Micr. Soc. Lond. in Quart. Jour. of Micr. Sci., Vol. IV, (1856) pp. 55-60.

It is in this paper that Mr. Wenham insists on making homogeneous contact with the slide and the top of the paraboloid. It will be noticed that in this paper he speaks of Opaque Objects, while in the paper of 1850 he speaks of Transparent Objects. By reading the two papers it will be seen that many of the objects mentioned in the two papers are identical. This gains an explanation from the fact that he has apparently given up the notion that the objects were visible by their own "radiated" light, but by the light they reflect to the microscope. Consequently he represents (Fig. 4) the light from the condenser going to the cover-glass and being reflected from it down upon the object and he says that it makes the most perfect kind of a Lieberkuhn reflector. One can see instantly that when homogeneous immersion objectives are used there can be no total reflection from the cover.

WOOD, ROBERT W.

1911. Physical Optics. New and Revised Edition, 1911.

On p. 373, he discusses, "Penetration of the disturbance into the second medium," and shows that going back to the time of Newton and Fresnel, it was known that while there was total reflection, the light seemed to pass for a minute distance into the rarer medium. This explains why one may get a brighter dark-field picture than is expected if objects are in optical contact with the slide.

WRIGHT, SIR A. E.

1907. Principles of Microscopy, being a handbook to the microscope. London and New York, 1907.

The writer has found this book the best and most thought-provoking of any that has been published on the microscope during the last 50 years.

A NEW BLADDER FLUKE FROM THE FROG*

BY
JOHN E. GUBERLET

Bladder flukes have been reported a number of times from North American frogs but as yet very little work has been done on these forms in this country. The European species, however, have received more attention and their complete life histories have been worked out. In North America the studies on frog bladder flukes have been carried on by only four authors, namely Leidy (1851), Bensley (1897), Stafford (1902, 1905), and Cort (1912). The localities from which these were reported are Toronto, Canada; Rice Lake, Ontario, Canada; Urbana, Illinois; Bemidji, Minnesota; and North Judson, Indiana. The writer has at hand another species of frog bladder fluke from *Rana catesbiana* taken at Stillwater, Oklahoma.

In view of the fact that Cort (1912) has given a thorough review of the literature as well as a discussion of the nomenclature of this group, it is unnecessary to take up the history of the literature any farther at this time. The frog bladder distomes have been grouped into two genera by Looss and called *Gorgodera* (1899) and *Gorgoderina* (1902). The basis for this classification is on the number of testes which these animals have. The genus *Gorgodera* has nine testes while *Gorgoderina* has two. Of the latter genus there are known from North America three species, namely *Gorgoderina simplex* Looss, *G. translucida* Stafford and *G. attenuata* Stafford. Of the former genus there have been two species described, namely, *Gorgodera amplicava* Looss and *G. minima* Cort. The writer adds another species to the genus *Gorgodera.*

GORGODERA CIRCAVA NOV. SP.

In the summer of 1918 the writer found in the urinary bladder of a large bull frog (*Rana catesbiana*) twenty trematodes (Figs. 1 and 2) which belong to a new species of the genus *Gorgodera.* In the early part of the summer of 1919 another bull frog yielded two specimens of the same species of trematode. These forms were so firmly attached to the wall of the urinary bladder by means of the acetabu-

*Contribution from the Parasitology Laboratory of the Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma.

lum that it was necessary to tear the bladder apart in order to make the worms release their hold. The worms were killed by dashing hot corrosive acetic over them when they were well extended. In this way they were only very slightly contracted when killed.

It was thought at first that this form belonged to the species *Gorgodera amplicava* Looss. Unfortunately, specimens of this species could not be obtained for comparison. From a study of the descriptions of *G. amplicava* in the literature on bladder flukes it was concluded that the species were not the same. The only other species of this genus known in North America is *Gorgodera minima* Cort. That species is much smaller than the one to be described here. The European forms are all much larger than any of the American species of *Gorgodera*.

This species of distome is similar in activity and habit to the others of this genus. The anterior portion of the body is very active and moves about freely while the posterior region is less active but not sluggish. The cuticle of the anterior part of the body is marked with minute longitudinal striations. These markings extend to or slightly beyond the acetabulum. The part of the body which is anterior to the acetabulum is cylindrical but becomes flattened near the acetabulum while the posterior portion is somewhat flattened and rather opaque. The opacity extends from the posterior end forward to the region of the ovary. That portion of the body occupied by the ovary, vitellaria and acetabulum is fairly transparent.

The length of the animal varies from 2.5 to 3.75 mm. with a width of .5 to .65 mm. in the region posterior to the ventral sucker. This form appears to be considerably smaller than *Gorgodera amplicava* which has a length of 3 to 5 mm., and larger than *G. minima*, that form being 1 to 2 mm. in length. The individuals which measure 2.5 mm. in length have large numbers of eggs in the uterus while in those of the larger size this organ is entirely filled throughout giving it the appearance of being a mere egg sac. In individuals which are less than 2.5 mm. in length no eggs are developed.

The ventral sucker ranges from .60 to .75 mm. in diameter and is surrounded by a distinct circular sheath 0.05 to 0.135 mm. in width (Figs. 1 and 2, vss). This circular sheath around the acetabulum is very marked and is a rather distinct characteristic in this form. Therefore, I wish to propose the name *Gorgodera circava* for this species.

The sheath around the sucker forms a distinct space or cavity between the wall of the sucker and structures of the body (Fig. 7). Small muscle bands (Fig. 7, mb) bind the tissues of the body to the ventral edge of the sucker. There are also a few muscle bands and connective tissue fibers extending across the cavity which connect the sucker with the internal parts of the body. From the external appearance of a normal animal the sheath is only slightly apparent from a side view and appears only as a slight bulge around the sucker. In an animal with both ends curved ventrally the sheath forms a distinct fold around the acetabulum (Fig. 4). The ventral sucker with the circular sheath produces a structure from .65 to .8 mm. in diameter, which is somewhat broader than the greatest breadth posterior to the sucker. The oral sucker has a diameter ranging from .30 to .37 mm. with an average of .33 mm. for ten specimens. The ratio of the oral sucker to the ventral sucker ranges from 1.8:1 to 2.3:1 with an average for ten specimens of 2.1:1. As stated by Cort (1912:162) the acetabulum of *G. amplicava* is 2.5 to 3 times the size of the oral sucker. Therefore, *G. circava* is different in this respect.

The mouth is situated in the oral sucker and appears as a triangular orifice in the posterior part of the sucker. The esophagus (Fig. 1, e) is a short narrow tube 0.14 mm. in length and 0.03 mm. in width. The intestinal ceca (Fig. 1 and 2, i) are about 0.055 mm. in width and are dorsal extending from the esophagus to within a short distance of the posterior end of the body. They are widely separated to give room for the reproductive organs which lie between as well as ventral to them. The ceca are dorsal and lateral to the testes. Some folds of the uterus pass to the lateral margins of the body and lie outside the ceca.

The reproductive system of *Gorgodera circava* is similar to that of the other species of this genus. The principal differences lie in the relative size and shape of parts, such as the number of vitellaria, shape of ovary, seminal vesicle and ejaculatory duct. There are nine testes, five on the same side with the ovary and four on the other. They are irregular in shape and the anterior ones are somewhat larger than those posterior. The shapes and sizes of the individual testes vary in different individuals but in general those which are anterior are proportionately broader than those posterior. With one exception the testes range about 0.23 mm. in length, 0.14

to 0.17 mm. in breadth and 0.22 mm. in thickness. The testis which is most posterior is usually much smaller than the others, measuring about 0.17 mm. in length by 0.12 mm. in breadth and 0.21 mm. thickness. The testes on either side are connected by minute tubules. From the dorso-anterior edge of the anterior testis on each side arises the vasa efferentia (Fig. 5, ve). These tubules extend anteriorly and unite in the region of the vitellaria to form the vas deferens which passes forward to the vesicula seminalis (Fig. 3, ves). The vesicula seminalis is a large pyriform sac dorsal to the anterior edge of the ventral sucker. It has a length of 0.15 to 0.2 mm., breadth of 0.14 mm. and thickness of about 0.15 mm. The shape and size is somewhat modified according to the degree of expansion or contraction of the worm. The vesicula seminalis is entirely filled with sperm cells. From the dorso-anterior edge of the vesicula seminalis the ejaculatory duct (Fig. 3, ed) arises and curves ventrad for some distance and then extends forward to the common genital pore (Fig. 3, g). This duct has a total length of 0.16 mm. and in the proximal region has a diameter of 0.015 mm. Around the distal portion of the duct are grouped the prostate glands (Fig. 3, p), a group of unicellular gland cells. In this region the ejaculatory duct is much enlarged forming a large pouch (Fig. 3, ep), or lumen in the midst of the prostate gland. This pouch or enlargement of the duct is 0.07 mm. in length and 0.05 mm. in diameter. The ejaculatory pouch as well as the duct is filled with sperms.

The vitellaria (Fig. 2, v) are immediately posterior to the ventral sucker and anterior to the ovary. They are made up of two groups of six to eight follicles each. One group lies toward each side of the animal and they are connected by a transverse vitelline duct. This duct becomes enlarged to form the vitelline reservoir in the median line of the body (Fig. 6, vr). From the dorsal surface of the vitelline reservoir arises a small median vitelline duct (Fig. 5, vd) which passes dorsal into Mehlis' gland where it unites with the ootype.

The ovary is a distinct three-lobed structure 0.27 mm. in length, 0.24 mm. in breadth, and 0.21 mm. in thickness. This organ lies toward the ventral side of the body. It may occur on either the right or left side as about half of the specimens studied showed it on one side and the other half on the other. The oviduct arises from the dorsal surface of the ovary as a funnel-shaped structure with the broad part of the funnel attached to the ovary. It extends

dorsad for some distance as it becomes narrow and then curves laterally or anteriorly, after which it enlarges immediately into the fertilization space (Figs. 5 and 6, f). It then becomes narrow again and passes forward near the dorsal surface of the animal to Mehlis' gland (Figs. 5 and 6, m) where it changes into the ootype. Mehlis' gland is a small group of unicellular gland cells located between the posterior edges of the vitellaria and dorsal to the transverse vitelline duct. Laurer's canal (Fig. 5 and 6, l) passes from the proximal region of the oviduct between the fertilization space and the ootype and makes a slight lateral curve. It then goes anteriorly and dorsally to the point where it opens on the dorsal surface of the body either dorsal or lateral to the ovary.

In passing from the ootype the uterus curves ventrad and bends back on itself (Fig. 5 and 6, u) in the median line of the body and goes posteriorly between the testes and finally reaches the posterior extremity of the body, where it fills with its numerous coils the region of the body posterior to the ovary and testes. The coils of the uterus become filled with eggs. Small masses of sperm cells are scattered throughout the coils of the uterus. The uterus finally emerges from the mass of coils in the region of the anterior testes (Fig. 2) and extends forward ventral to the ovary and vitellaria, passes dorsal to the ventral sucker and ventral, or slightly lateral to the vesicula seminalis to the genital pore (Fig. 3).

The eggs of *Gorgodera circava* increase in size as they develop and pass from the ootype to the genital pore as in other species of the bladder flukes. In this case only the eggs in preserved specimens have been studied and no doubt there has been some shrinkage through the process of preservation. The eggs at the ootype measure about 0.016 mm. in length by 0.013 mm. in breadth; at the posterior end in the coils of the uterus 0.025 mm. in length by 0.019 mm. in breadth; while at or near the genital pore where they contain fully developed embryos, about 0.030 mm. in length by 0.023 mm. in breadth.

The chief differences between the American species of *Gorgodera* lies in the size and shape of the animals; the structure, size and ratio in sizes of suckers; and the shape and relationship of the reproductive organs. *Gorgodera minima*, described by Cort (1912) is the smallest of the three species, it being 1 to 2 mm. in length and its acetabulum is 1.6 to 2 times the size of the oral sucker. *Gorgodera amplicava*

first described in this country by Bensley (1897), and reviewed by Stafford (1902), and again compared with *Gorgodera minima* by Cort (1912), is considerably larger being 3 to 5 mm. in length and its acetabulum is 2.5 to 3 times the size of the oral sucker. *Gorgodera circava* is 2.5 to 3.75 mm. in length and the acetabulum ranges from 1.8 to 2.3 times the size of the oral sucker. The acetabulum is also surrounded by a distinct circular sheath which is a distinctive characteristic of this species. In *Gorgodera circava* the vitellaria are composed of six to eight follicles in each group while *Gorgodera amplicava* has eight to ten in each and *Gorgodera minima* has nine to eleven. The ovary of *Gorgodera circava* is a distinct three-lobed structure while in *G. minima* it is only slightly lobed and in *G. amplicava* it has three to five irregular lobes with smaller or secondary lobes. The presence of the ejaculatory pouch in *Gorgodera circava* is another structure not found in either of the other species. The differences in the reproductive organs and the presence of the circular sheath around the acetabulum clearly sets *Gorgodera circava* off from the other species.

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LEIDY, J.
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1902. The American Representatives of *Distomum cygnoides*. Zool. Jahrb., Syst., 17:411-424.
1905. Trematodes from Canadian vertebrates. Zool. Anz., 28:681-694.

EXPLANATION OF PLATE XIII

All drawings made with the aid of camera lucida.

Fig. 1. Dorsal view of *Gorgodera circava*, X35.

Fig. 2. Ventral view of *Gorgodera circava*, X35.

Fig. 3. Reconstruction from sagittal sections showing ends of reproductive organs and genital pore, X130.

Fig. 4. Outline drawing of small specimen which is bent ventrally at both ends causing the acetabular sheath to form fold around sucker, X35.

Fig. 5. Reconstruction of female genital organs from sagittal sections, X120.

Fig. 6. Reconstruction of female genital organs from frontal sections as seen from dorsal view, X120.

Fig. 7. Sagittal section through ventral sucker to show ventral sucker sheath, X35.

Abbreviations

e	esophagus	*ov*	oviduct
ed	ejaculatory duct	*p*	prostate gland
ep	ejaculatory pouch	*u*	uterus
ex	excretory pore	*v*	vitellaria
f	fertilization space	*va*	vas deferens
g	genital pore	*ve*	vasa efferentia
i	intestinal ceca	*ves*	vesicula seminalis
l	Laurer's canal	*vd*	median vitelline duct
m	Mehlis' gland	*vr*	vitelline reservoir
mb	muscle bands	*vs*	ventral sucker
o	ovary	*vss*	ventral sucker sheath
os	oral sucker	*t*	testes

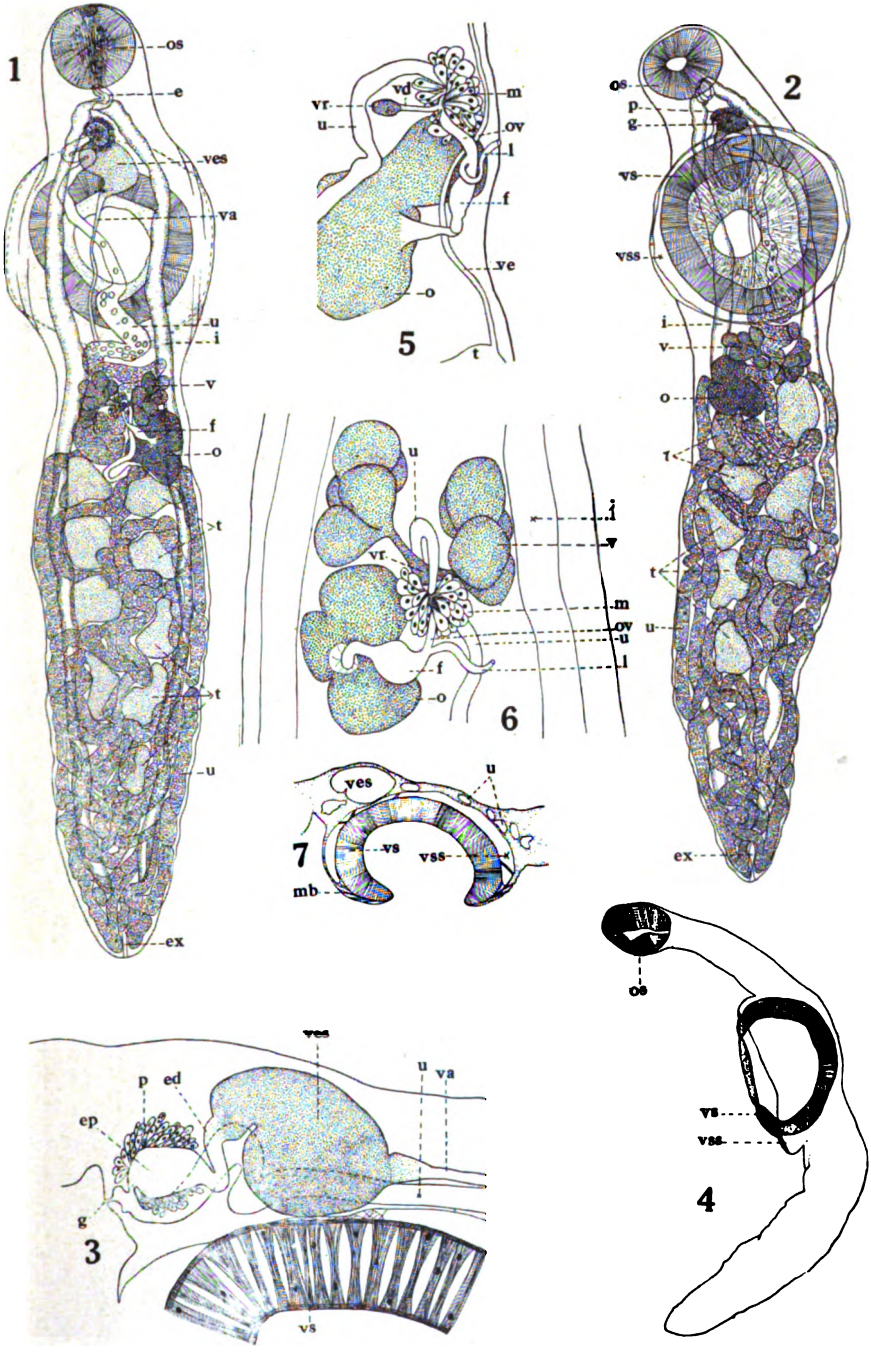

PLATE XIII

GUBERLET

LABELING ILLUSTRATIONS

BY
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Sometimes really good illustrations are spoiled by faulty or poorly made labels and not infrequently biological illustrators do not do sufficient labeling to make their illustrations clear. The thought is frequently expressed by good draughtsmen that labeling is difficult and is therefore to be avoided, or they say they wish that they had been born with the ability to letter drawings properly thus expressing clearly that in their own opinions their drawings are not labeled as they should be. With these thoughts in mind it seems not amiss to outline the following methods that may be used for labeling biological illustrations.

It frequently becomes necessary to indicate separate parts on an illustration, hence it becomes necessary to label the drawing. This may be done in a variety of ways. If the person making the illustration has enough ability he may make the lettering free handed. Another method is to select letters or figures from printed matter. These may then be cut out and pasted on the illustration in the proper place. Still another method is to buy the cut-out letters and figures and paste these on the illustration. The sets desired may be written on the typewriter and cut out and pasted on the illustration. Labels may be printed directly on the illustration. And lastly guide lines may be drawn to the margins of the illustrations where they may be connected with type set up in the ordinary way when the illustration is printed. Regardless of the method of labeling selected care should be exercised to make the labels as neatly and accurately as possible. Care should also be exercised to see that the lettering is sufficiently large to stand the necessary reduction if the illustration is reduced.

The labels of biological illustrations are generally indicated in the following manner: (1) by Arabic numerals, (2) by the initial letters of the name of the parts of the object labeled, (3) by abbreviation of the names of the parts of the object labeled, (4) by a sequence of letters, and (5) by the full names of the parts of the object. In the first four methods it is necessary to print an explanation of the

labels. The fifth method requires no explanation. The method selected will depend upon the personal choice of the draughtsman. There is much to be said in favor of the fifth method provided there are not too many parts to label or the names are not too complicated. By this method the attention is not distracted by having to search through a long list of explanations. If it is preferred to abbreviate the names, then there is much to be said in favor of the second and third methods, for the initial letters and the abbreviation will indicate the real name of the part. If either of these methods is selected the explanations should be arranged alphabetically, so that it will not be necessary to look through the entire list to find the explanation for any abbreviation.

The labels may be placed at the margin of the illustration or directly on the illustration. The method we select will depend to a great extent on the nature of the illustration. If the parts are large enough so that the labels may be placed directly on the part without obscuring any details this is perhaps the best method. For many illustrations, however, this is not possible. The labels should then be placed at the margin. This latter method necessitates the use of guide lines. Guide lines may be simple straight lines or they may be brackets. Brackets are used to indicate areas of considerable extent which could not be indicated by a single line. Instead of the bracket, two lines drawn at an angle to each other may be used. The straight lines may be solid, dotted or dashed lines. If a dashed or dotted line is used care should be taken to get the dots of uniform size or the dashes of uniform length. As a general proposition guide lines should be straight and should run parallel to the main margin of the drawing if possible. On very light drawings the guide lines should be black. If the guide lines run over alternate dark and light areas it is advisable to use a double line, one part being black and the other part white. Care should be exercised to keep guide lines of uniform thickness. This may readily be accomplished by using a ruling pen. Ordinary liquid India ink may be used for black lines and any good grade of Chinese white for white lines. Care should be taken not to make the lines so thick that they will look unsightly when the drawing is complete. On the other hand, the lines should not be so delicate that they will not stand the necessary reductions.

Cut out letters or figures which are gummed on the back may be purchased in a wide variety of styles and sizes. These are very desirable for labeling drawings especially if the correct size and a suitable style are selected. Care should be taken to see that the separate letters are pasted in a straight line. This may readily be accomplished by drawing a faint pencil line to indicate the bottom of the letters, and then bringing the letters to this line. Cut out letters have the advantage that they may be pasted directly on the illustration, and will obscure only a minimum amount of detail.

Labels may be written on the typewriter using a good black record ribbon. In making labels on the typewriter only a new ribbon should be used and the type should be thoroughly clean so that good sharp impressions can be secured. These labels may then be cut out and pasted on the drawing as recommended above for printed labels. The chief difficulty with this method is that the labels are too small unless the illustration is not to be much reduced in reproduction. Type written labels may be enlarged by making a negative from them by any of the enlarging methods, and then printing a positive from this negative on a smooth gaslight paper. This method is advocated chiefly where we need a large number of labels of the same kind.

Labels may be selected from printed matter and pasted on the drawing. It is necessary to bear in mind the amount of reductions that will take place in reproducing the drawing. The ordinary book type is about 9 points, therefore if the drawing is reduced to one third the original labels should be 28 point. If the drawing is reduced to one fourth the original label will have to be 36 point. It is usually difficult to find letters of sufficient variety in these sizes. Special labels may be set up by the printer but this is usually a very expensive method. Plate XIV shows the various sizes of printed letters and may be used to determine the size of letters that it is necessary to use. Thus if the drawing is reduced to one third labels the size of 36 point will appear as 12 point or 18 point will appear as 6 point, etc. The various sized letters on this plate may be traced on thin tracing paper in India ink and pasted on the drawing.

Hand Printed Labels from Rubber Type

Labels may also be printed by hand from rubber type directly on the illustrations. For printing labels from rubber type we will

need a set of rubber type of the proper size, holder to hold the type, and a stamp pad filled with faint blue ink. The proper combinations of letters are set up in the holder, bearing in mind that the type are inverted and reversed. The type in the holder are then stamped in the pad and then on the drawing. The labels are then traced over with India ink. It is necessary to trace over labels made with a rubber stamp because the margins are not clear cut. The advantage of using the pale blue ink is that if the illustration is reproduced by ordinary photographic processes the blue will not show and need cause no trouble if slight errors are made. Rubber type may be secured in a variety of styles and sizes. A neat legible style should be selected and a size selected so that it will stand the necessary reduction. Sets of complete alphabets may be purchased or separate stamps may be produced from dealers in office supplies. The former has the advantage that any label may be readily set up. The latter is preferable if many labels of the same kind are to be printed at one time. The holders for rubber type are usually supplied with the sets of type. These holders are convenient and since they are adapted to the size type with which they are supplied they leave little to be desired. Stamp pads for this purpose should be inked with a faint blue ink as this color is not very active, photographically it does not bother the engraver. After the labels have been stamped with the faint blue ink they must be finished up with black India ink. This requires a fine pen and a little attention to details, but can be done with considerable rapidity after a little practice.

Hand Printed Labels from Metal Type

Labels may also be printed from the regular metal type of the printer very much as the rubber type is used. To print labels from metal type we will need some black printers ink, a font of type of proper size, a holder for the type and a compositor's roll.

The ink used for hand printed labels of the metal type is the regular black printers ink. This usually requires thinning to work properly. Benzine, gasoline or xylol may be used for thinning the ink. For this purpose the ink is placed on a piece of glass and the solvent is added drop by drop while the ink is worked with a spatula, until it is of the proper consistency. Experience soon teaches when the ink is of the proper consistency. When it is thought that the ink is properly mixed a small amount of the prepared ink is spread on the

compositor's roll and the type in the holder is stamped on the roll and printed on a piece of paper. If the ink is in the proper condition and has been properly spread on the roll enough will adhere to the face of the type to make a good label. If the ink is too thin it will spread when we attempt to print a label. If it is too thick not enough of it will adhere to the face of the type to print a good label.

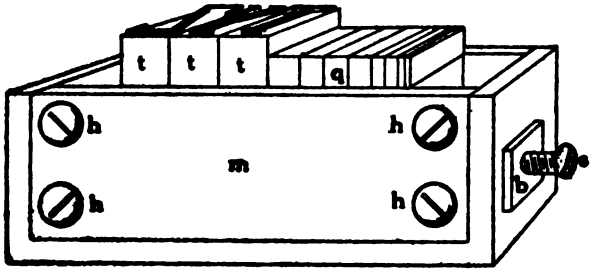

Fig. 1. Type holder for printing labels.

b, bur of the stove bolt which is soldered to the type box.
h, heads of stove bolts used to clamp the movable plate against the type
m, movable plate
q, quads which are used to fill out the line of type
s, stove bolt used to clamp the type in the line
t, type

The type holder (Fig. 1) used for hand printed labels is a shallow box made of brass. The height of the box is a little less than the height of the type. One side of the box is movable and is fastened to the opposite side by means of four set screws. This is used to lock the type in rows. One end of the box has a set screw which is used to lock the type. This holder is used very much as an ordinary rubber stamp is used. If only one figure or letter is to be printed in each label it is not necessary to use the type holder, but the individual types can be held in the hand very readily.

Type is set up in an inverted and reversed position. Each piece of type has little grooves called the nicks which indicate when the type is in a proper position. As soon as a line of type is set up a glance will reveal whether all of the characters are in the proper position. The characters to be used are picked out from the type box one at a time and placed in the holder which is held in the left hand in an inclined position so that the type will lay against the fixed side of the holder. As soon as the label is completely set up the rows of type are locked in place by tightening the set screws

which fasten the movable side and the end. At first the set screws are only set tight enough to hold the type firmly in place. A soft wooden block is then placed on the face of the type and the type is leveled up by pounding with a light hammer. The properly set up label is firmly pressed on to the inked compositor's roll and the inked type is then stamped on the illustration. Care being taken to press the type down firmly at all points without allowing it to move.

The advantages of hand printed labels are that they are very neat and accurate and that they may be made by any one without previous experience. The chief disadvantage is that it is somewhat laborious to set up the type. But with the large sized type used in printing labels for illustrations this is not a very large item. It must be remembered that it requires several hours for the printers ink to dry and care must be exercised in handling the illustrations or the labels may be ruined.

Free-Hand Lettering

Occasionally it is not possible to letter an illustration by any of the methods given above, in that case it is necessary to have recourse to free-hand lettering. Free-hand lettering is a special kind of free-hand drawing by means of which the draughtsman learns to draw the design of letters neatly and rapidly. There are no special tricks of the trade about lettering that cannot be learned by the biologist who will conscientiously try to master the subject.

The first consideration in free-hand lettering as in other kinds of free-hand drawing is to get the main proportions. After the main proportions are secured the details are added. The more important details are added first then the finer and finer details until the lettering is complete. Just as the individual letters are found to vary one from the other so in printing a line of lettering it will be found necessary to space the letters carefully with reference to each other, otherwise the lettering will not have a neat appearance when finished. In lettering each letter is influenced by the letters on each side of it so that no general rule can be laid down which will make it possible to always place a letter the proper distance from its neighbors. The only rule that can be given is that all letters should seem to have the same amount of space allotted to them. Obviously this amount of space will vary with the different letters and with the letters on each side of it. Thus a capital I requires less space than a capital M.

Then, too, a capital I will require more space if placed between a capital M and a capital N than if placed between a capital E and a capital T because these letters have a great amount of free space whereas the M and N have practically no free space. The letters in each word must be studied, therefore, in order to determine the proper spacing. Trials should be made in order to see just what spacing looks the best. If this is done critically gradually a proper conception of proper spacing of letters will be acquired.

The usual error made by beginners is to space the letters too far apart. Letters look better when they are crowded well together.

The letters in any design that is to be treated in free-hand lettering should be sketched in with a pencil complete before finishing up any of the letters. This is done in order to insure a proper balance of the words with each other and a proper spacing of the letters. In sketching letters it is usually advisable to draw two faint lines one for the top and the other for the bottom of the letters. Sometimes it is advisable to divide this space by a third line so that certain letters may be carefully drawn with rapidity. After the base lines are drawn the letters are sketched in spacing each letter with reference to the other letters, and indicating at first only the general outlines of the letters. Corrections should be made until the whole has a well balanced appearance. Some letters may then be carried forward and the principal minor details indicated, making any necessary corrections in the other letters in the line to maintain the balance. After experience has been gained letters may be sketched in ink free-hand, especially such forms as the Gothic. And any one having considerable lettering to do should practice this form of lettering, but the art of lettering is soon lost unless it is used day by day.

After the pencil sketch of a line of lettering is finished the letters may be finished with India ink on drawings or with black water color or oil color on paintings. In doing this the borders of the main letters are finished first and then the borders of the finer details; the body of the letters being filled in last of all. Care must be taken in finishing up the borders not to exceed the limits penciled and to keep all straight lines straight and all curved lines a true curve. In filling in the body of the letter care must be taken not to allow the color to run over the borders that have been finished. In finishing large letters a ruling pen and a straight edge may be used for the longer

straight lines on the borders of the letters but on the smaller letters and for all fine details a pen must be used free hand.

The idea is prevalent that the forms of letters are fixed but nothing is farther from the truth. There are certain broad general styles of letters such as the Roman and Gothic but the variations in these styles are as many as there are draughtsmen. A few of the more important styles are discussed below and plates showing standard letters are given, not with the idea that these should be copied slavishly but that these designs may be helpful in producing letters for drawing and may serve to indicate the main styles. All letters occur in two forms, capitals usually called caps and small letters called lower case. If there are only a few letters in a group as in the abbreviated signs used to label parts of a drawing either caps or lower case or both kinds of letters may be used, but if there is a series of words it is better to use lower case letter throughout as we are used to reading words printed in lower case types. Words in lettering should be well separated so that there is no doubt as to the limits of the separate words. The rule is that the words should be separated at least by a space equal to that occupied by the widest letter and slightly more space would be better.

The Gothic letter is the simplest letter because it is formed of lines of a uniform thickness throughout. Gothic letters exist in two forms, a vertical Gothic and an inclined Gothic. In the vertical Gothic alphabet the main axis of the letters is vertical and since the lines are all of a uniform thickness it is a fairly easy alphabet to letter in a free-hand manner. For convenience of discussion the letters are divided into the following groups, (1) letters composed of straight horizontal and vertical lines, (2) letters composed of horizontal or vertical lines with diagonal lines, (3) letters composed of straight and curved lines and (4) letters composed of curved lines only. Furthermore it is convenient to define a full bodied letter as a letter occupying as much horizontal as vertical space. For purposes of analysis the letters are drawn on cross section paper each letter occupying a vertical distance of five units. The thickness of the stroke is only 2/3 of a unit.

In the first group of letters we have the capitals E, F, H, I, L and T and the lower case letters i, l. In the capital E it will be noted that the letter is not a full-bodied letter as the foot occupies only 4½ units and the cap only 4 units. The tongue of the letter occupies

only 2½ units and is placed only slightly higher than the middle. The capital F is identical with the E except that the foot is omitted. Care should be taken not to extend the cap too much or the letter will look top-heavy. The capital H occupies about 4 units as otherwise it looks too broad. The tongue is placed on a level with the tongue in the E and F, that is slightly above the middle. The capital I needs no comments as it is simply a straight line with a thickness of 2/3 of a unit. The foot of the capital L is about 3½ units in length to prolong it makes it appear unwieldy. The full bodied lower case letter occupies only three-fourths the space allotted to the full bodied capitals and the width of stroke is only a half unit. The small lower case letters occupy only two-thirds of the vertical space occupied by the large lower case letters. Therefore the body of a small lower case letter like i would occupy only one-half of the vertical space allotted to a capital letter, hence 2½ units the dot being placed one full unit above the top of the letter. The lower case l would occupy ¾ of the vertical space allotted to a capital.

To the second group belong the capitals A, K, M, N, V, W, X, Y and Z, and the lower case letters k, v, w, x, y and z. The capital A occupies the full width of five spaces below and slopes to the top line uniformly on both legs. The top does not end in a sharp point but in a point that is about one-half unit wide. The tongue of the A is placed about one and one-half units above the base line. The capital K is somewhat difficult as it is composed of two diagonal lines at different angles. The top diagonal is usually placed about three and one-half spaces from the vertical stroke and at such an angle that if it were projected the lower border of the diagonal would strike the base line one full unit to the left of the vertical stroke. The lower diagonal is placed four units from the vertical stroke and at such an angle that its top border projected would strike the top of the vertical stroke. The capital V is simply the capital A inverted and the tongue omitted. The capital M is simply the V with two vertical strokes added on each side. Note that these vertical strokes end in their full width and not reduced as in the case of the top of the A and the bottom of the V. The capital N consists of two vertical strokes four units apart connected by a diagonal running from the top of the left hand stroke to the bottom of the right hand stroke not the reverse as is frequently seen in lettered signs. The diagonal is placed at such an angle that the vertical strokes will end in full

width on both the base and top limiting lines. The capital W may be considered as two V's contracted to occupy only four spaces each and united so that the apex of the jointed diagonals shall occupy only half a unit each. The capital X is simply two diagonals which cross each other in the center. This letter is therefore a full bodied letter. The capital Y is composed of two arms which are six units apart and run at such an angle as to unite two and one-half units from the base line. The foot of the capital Z is four and one-half units long and the cap only four units long. The cap and the foot are connected by a diagonal placed at the proper angle. In the lower case letters the stem of the K occupies the full vertical unit for lower case letters and the diagonals of the letter bear the same relation to each other that they do in the capital K but they are reduced to one-half. The lower case v, w, x and z are the same as the caps except they are reduced to one-half. The lower case y is the same as the lower case v with the right diagonal extended below the base line, the full length allotted to lower case letters.

In the third group we have the capitals B, D, J, P, R and U; and the lower case letters a, b, d, e, f, g, h, m, n, p, q, r, t, and u. The capital B may be considered as a capital E with the ends of the cap and the foot connected to the tongue by arcs of circles. It will be noted that this makes the top part of the letter somewhat smaller than the bottom. The capital R may be considered as a capital F with the cap and tongue connected as in the B and a tail added to the lower part. The tail of the R should extend beyond the top part of the letter at least a full unit otherwise the letter will look top-heavy. The P is similar to the R without a tail but the top part of the P is made longer by dropping the tongue about one-half unit below its position in R. A capital D is produced by using a foot and cap similar to the foot and cap in the capital B and connecting these two horizontal lines by a regular curve. The capital J and U are similar to each other save that the J has a single vertical arm and the U a double arm. The J is somewhat narrower occupying only three and one-half units whereas the U occupies about four and one-half units. The vertical arms are in each case about three and one-half units long. In the lower case letters b, d, p and g all have the same form and the q is simply the g with the stem turned to the left to distinguish these two forms; and the a is quite similar but with a shorter stem. The lower case u may be taken as the type of another

group of letters. It is essentially like the capital U with the right arm extended to the base. The lower case n is simply the u turned upside down and reversed and the m is simply two ns contracted slightly and united. While the lower case h is simply the n with the left side prolonged the full length of lower case letters. The lower case f, j, r and t are essentially vertical lines with short curved tails added. The capitals O and Q are essentially complete circles which extend slightly above the top line and slightly below the base line. The capitals C and G are parts of circles and offer no special difficulties. The capital S is composed of two curves joined by a third curve and is one of the most difficult letters to handle. The upper and lower limbs are arcs of ellipses whose major axes lie in horizontal planes with the major axis of the upper ellipse slightly shorter than the major axis of the lower ellipse and with their minor axes about in the ratio of two to three. The lower case o, a and s are essentially the same as the corresponding capitals and lower case c is similar with a horizontal line across the upper two-thirds of the circle.

The Gothic numerals may be taken as standard just as we took the Gothic letters. It will be noted that the numerals 1 and 4 are the only ones composed of straight lines only. The numeral 1 is a straight line ½ unit wide, the numeral 4 has a total width of four units and is drawn so that the horizontal tongue is one and one-half units above the base line. The numerals 3 and 8 are essentially the same being composed of two broad ellipses joined together the upper ellipse having a shorter major axis than the lower ellipse. The minor axes of the two ellipses bear a relation of about 2 to 3 to each other. The numeral 0 is merely a flattened ellipse with a major axis of 5 units and a minor axis of 4 units. The numerals 6 and 9 are the same being simply placed in different positions. It will be noted that they are essentially the same as the numeral 0 except for the formation of the small ellipse at the bottom of the 6 and the top of the 9. Note further that the tail of the 9 is somewhat expanded being near the base line and that the tail of the 6 is somewhat contracted being near the top line. This preserves the proper balance. The numeral 5 is essentially the same as the 6 except that the tail is composed of a straight vertical and horizontal line. The top is somewhat contracted to preserve the proper balance. The numeral 7 is four units wide with the curved vertical stroke ending on the base line about one unit to the right of the point where the horizontal line starts on the

top limiting line. The numeral 2 is the most difficult in the whole series as it consists of a compound curve. The top curve is somewhat like the top curve of 3 but is flatter and the bottom curve is more pronounced than the curve in the numeral 7.

The inclined Gothic is the vertical Gothic inclined at about 15° from the perpendicular. We need not make any special analysis of the separate letters as that has been done for the vertical Gothic. This is a favorite alphabet for draughtsmen who do a great deal of lettering as it can be done with great speed and if carefully done it looks neat and is very legible. For these reasons it is especially valuable for large amounts of labeling.

The Roman Gothic is in many respects a more pleasing alphabet than the Gothic. The basis of the letters is the same as for the Gothic but certain lines called body strokes are shaded by being made thicker, while some lines are made thinner than in the Gothic letter. The shaded or body strokes are usually made one unit wide and the hair lines are usually made one-half unit wide. Roman Gothic letters of widely different appearances may readily be secured by varying the width of the hair line. It should be noted that the curved body strokes are slightly thicker than the straight body strokes. Where curved lines join straight lines the union is made very gradually so that the eye cannot detect the point of union.

The Roman letter is a further modification of the Roman-Gothic by the addition of serifs to the strokes so that no lines end with a line of uniform thickness. This is the type of letter used in most printing and is the most difficult letter for the draughtsman to handle. However, it is perhaps the neatest appearing letter and should be used more extensively than it is at the present time. The separate letters need not be analyzed separately because they have essentially the same form as in the Gothic alphabet. The body stroke is usually considered as one unit in width for the straight lines and slightly wider for the curved lines. Sometimes variation in this standard is made for some special purpose. The hair lines vary greatly in different styles of this letter from lines as thin as they can be drawn easily to lines at least half a unit in width. By varying the widths of the hair lines, letters of quite different appearances may easily be secured. The serifs demand special attention and must be drawn neatly and accurately or they will ruin the appearance of the lettering. Horizontal serifs are usually about one unit in length and are con-

nected to the main stroke by a gentle curve which is made tangent to the main stroke and to the serif. Vertical serifs are usually made about one and one-half units long and are connected to the main stroke by a gentle curve which is tangent to the serif but not tangent to the main stroke. Exception, however, must be made in the case of the double serifs found on the tongue of the E and F, which are smaller than the other vertical serifs. In letters like E, s and Z in which are two vertical serifs the upper one is made slightly shorter than the lower one for the sake of appearance. In the capital J and some of the lower case letters it will be noted that curved lines instead of ending in straight serifs end in curved comma shaped marks called kerns. Instead of filling in the body strokes of Roman letters solidly they may be indicated by two hair lines. This makes a neat appearing letter and is useful for display titles but is difficult to execute and therefore seldom employed in labeling biological illustrations.

EXPLANATION OF PLATES

Plate XIV. Letters and figures of various sized type.
Plate XV. Vertical Gothic letters analyzed.
Plate XVI. Roman Gothic letters analyzed.
Plate XVII. Roman letters analyzed.

PLATE XIV METCALF

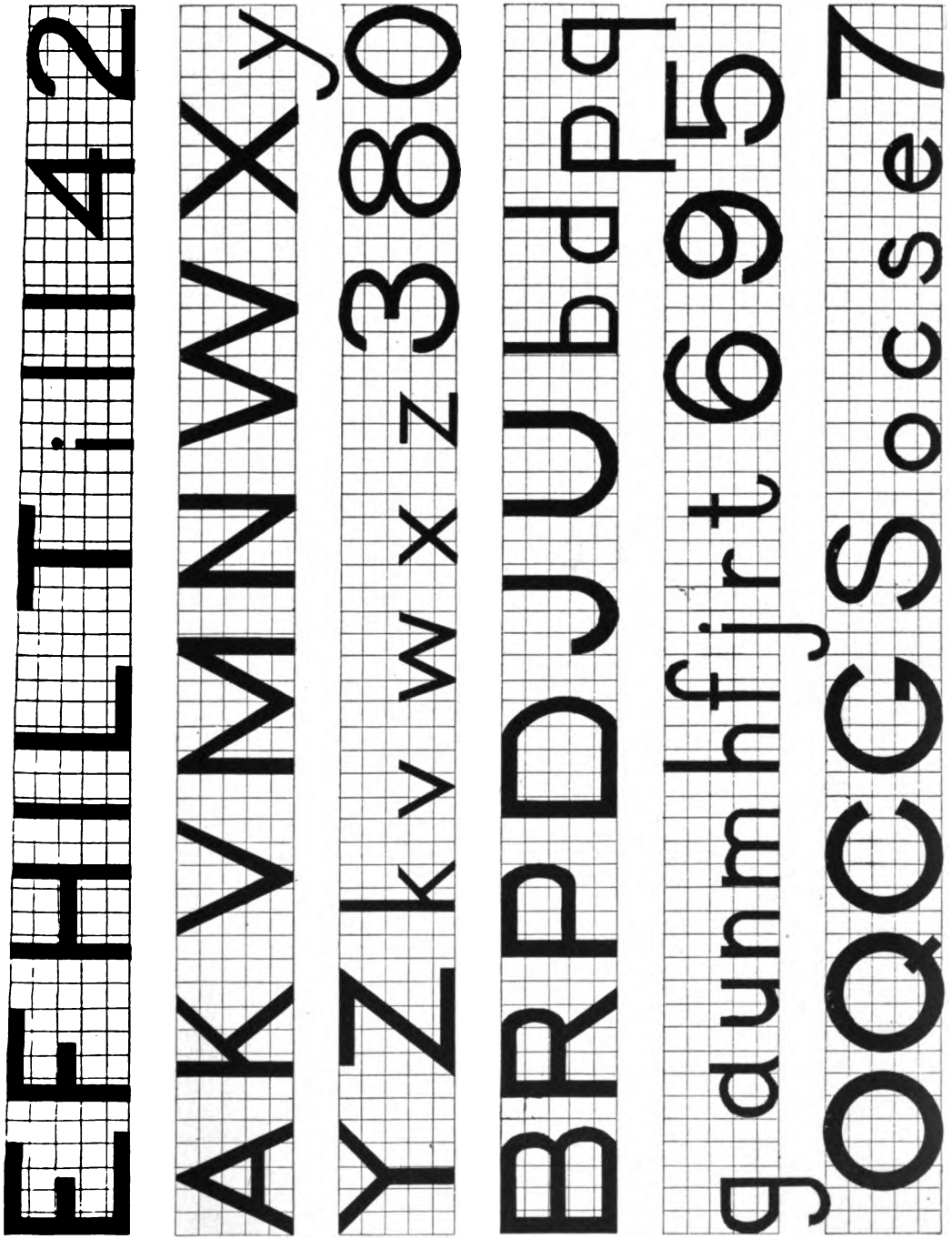

PLATE XV

METCALF

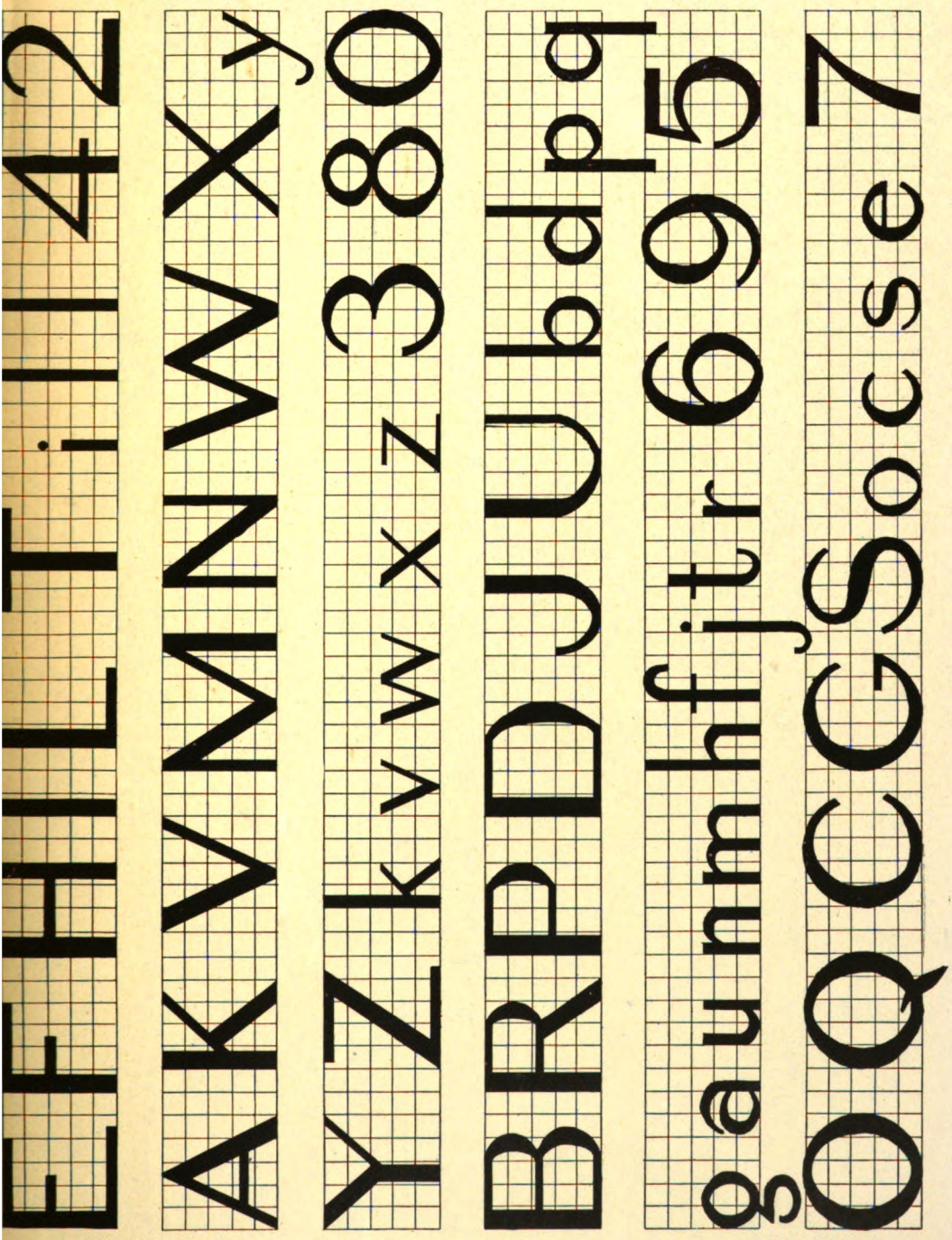

PLATE XVI

METCALF

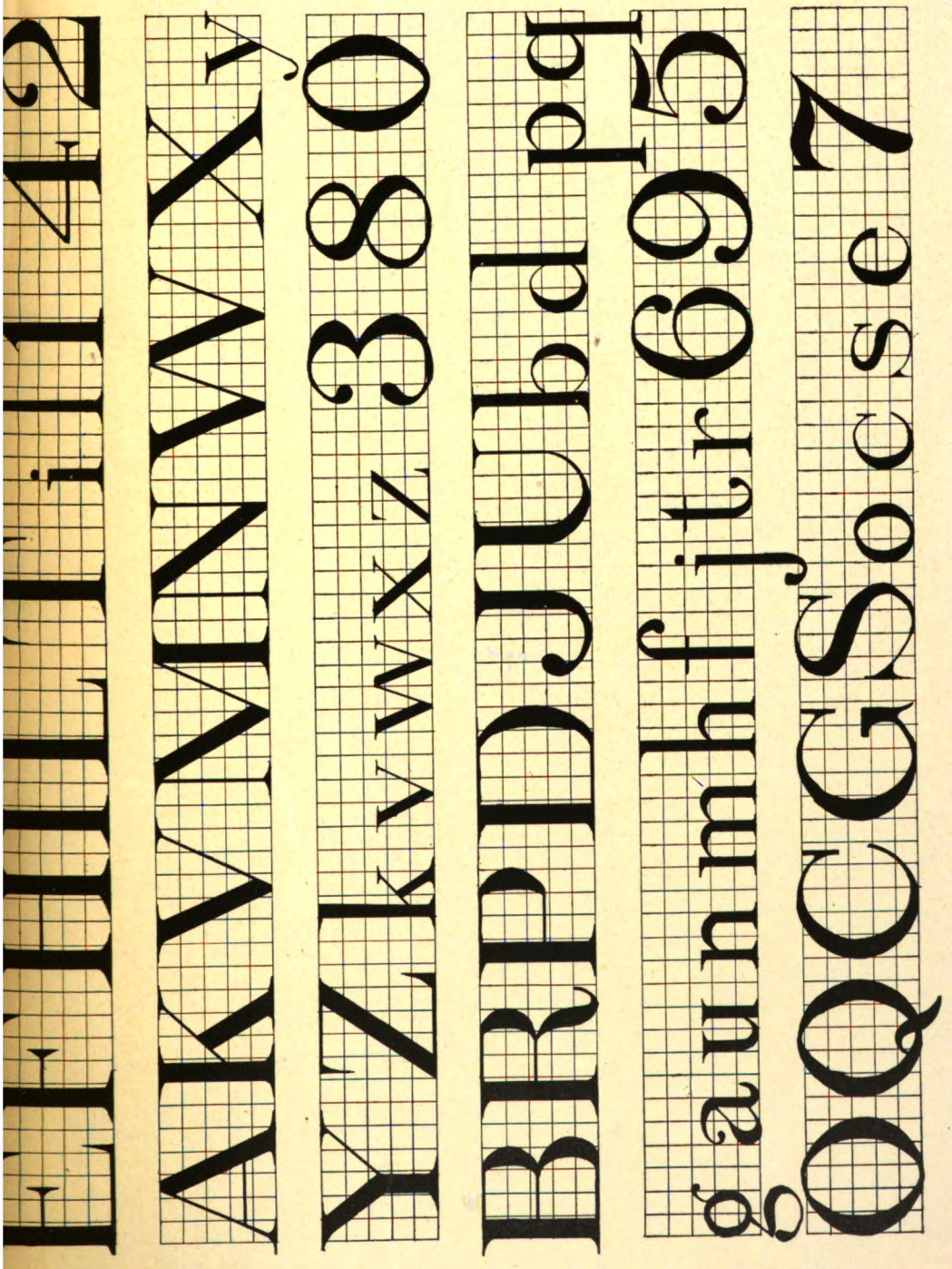

PLATE XVII

METCALF

DEPARTMENT OF NOTES AND REVIEWS

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In addition to these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

ENTOMOLOGICAL ABSTRACTS

Position of Micropterygidae.—Tillyard (1919, Proc. Linn. Soc. N. S. Wales, 44:95–136) has made an extensive study of the remarkable family of archaic moths, the *Micropterygidae*. Chapman (1917) removed the genus *Micropteryx* from the remainder of the family and proposed a new order, Zeugloptera, for its reception. Comstock (1918), on the other hand, removed the whole family *Micropterygidae* from the Lepidoptera and placed it as a new suborder of the Trichoptera. Tillyard finds no justification for either of these views. The proposed "Zeugloptera" is found to lack a single character not found in some other order. In all of the Micropterygidae M_4 does not occur as a separate vein of the forewing; the characteristic trichopterous wing-spot is lacking; the pupal wing-tracheation is complete; the scales are broad and possess numerous striae; and functional frenula are present. These characters definitely rule out the possibility of these insects being Trichoptera, and necessitate the conclusion that that they must be archaic Lepidoptera.

Micropterygidae.—Braun (1919, Ann. Ent. Soc. Am., 12:349–367) has also attacked the problem of the position of the Micropterygidae. A study of wing structure in the primitive Lepidoptera shows, according to this writer, that while the Micropterygidae stand close to the common ancestor of Lepidoptera and Trichoptera they are true Lepidoptera and have given rise to all of the remainder of that order by several divergent lines, one represented by the Nepticulidae, another by the Hepialidae, and a third "much branched line includes the frenate Lepidoptera, of which some members such as the Prodoxidae, Incurvariidae, etc., conserve some of the trichopterous characters of their ancestry and must therefore be regarded as the most primitive of the Frenatae."

Filariasis in U. S.—Francis (1919, U. S. Publ. Health Service, Hygienic Lab. Bull. No. 117) reports on a study of filariasis in Southern United States. *Filaria bancrofti* is the species concerned and one endemic focus has been located in this country at Charleston, S. C. Of 400 individuals examined in that city, 77 were infected with microfilaria, whereas of 1,470 examinations in nine southern cities outside of Charleston only 9 showed infection. The data indicated that cases outside of Charleston have derived the infection either from residence in Charleston or from residence at some place outside of the United States, as in Cuba. Transmission occurs only through the mosquito, but the certainty of the process is limited by the following facts: (1) No multiplication of the filaria in the mosquito; (2) The small number actually passing successfully through the mosquito; and the still smaller number which reach the lymph glands of man; (3) Male and female filaria must find lodgment in the same lymph gland of man in order that reproduction occur; (4) Infection of mosquitoes can occur only during a few hours before and after midnight; (5) The biting act of the mosquito only drops the microfilaria on the free surface of the skin of man whence it must penetrate the intact skin. The mosquito, *Culex fatigans*, was found to be the transmitter. The anatomy of the mosquito proboscis in relation to filaria transmission is discussed and the inward and outward courses of the filariae pointed out. The former is through the stilette bundle along with the ingested blood, while the latter is through the interior of the labium. Eight well executed plates, mostly in color, add to the value of the paper.

Polyembryony and Sex.—Patterson (1919, Journ. Heredity, 10:344-352) reports results of a study of the origin and development of mixed broods in polyembryonic Hymenoptera and the ratio in production of males and females. In 162 broods of *Copidosoma gelechiae*, 90 were female, 62 male and 10 mixed. The sex ratio was found to be approximately 3 females to 2 males. The great excess of females in four of the mixed broods suggested the possibility that both sexes might arise from a single fertilized egg. In *Paracopidosomopsis floridanus*, 1.7% of the broods were pure female, 11.3% pure male, and 87% mixed. The percentage of males varied from 0.06 to 72.07 and in over 58% of the broods less than 10% of the individuals in any brood were males. In *Platygaster rubi* not a single pure male brood was found. This, however, might be explained by the

prevailing conditions which make it unusual that an unfertilized female might escape. Only 6 of the 105 broods were pure female. In the 99 mixed broods, the number of females, in every brood, exceeded the number of males. In 53 broods only one male per brood appeared, 17 had 2 males each, and 13 had 3 each. The other broods showed a varying number, but not exceeding 10. That some mixed broods result from two parasitic eggs, one from a fertilized female and one from a virgin female, seems very probable but two diffiulties stand in the way of the exclusive application of this application, namely, (1) simultaneous emergence of individuals of a mixed brood, and (2) striking predominance of females over the males in the great majority of broods. A *Paracopidosomopsis* female, in about 66% of the cases, deposits two eggs in the host egg at a single oviposition, and in the majority of cases both eggs were found to be fertilized. A host egg mass of 28 eggs exposed to a mixed brood of parasites yielded 14 with 1 parasitic egg, 11 with 2 each, and 3 with 3 each. Eight of the 11 indicated two ovipositions, while 3 seemed to represent one oviposition. In each of the 3 remaining eggs the three parasitic eggs apparently represented different ovipositions. Therefore the two-egg explanation seems inadequate for the mixed broods of *Platygaster*. It is proposed that some of the mixed broods may result by one fertilized egg giving rise to both sexes through abnormal behavior of the two sex chromosomes during early cleavage, as for example, somatic non-disjunction in which certain blastomeres receiving but one x chromosome would produce male embryos.

Origin and Significance of Metamorphosis.—Crampton (1919, Bull. Brooklyn Ent. Soc., 14:33-40; 93-101) considers critically the problems of origin and significance of metamorphosis in insects. Presence or absence of metamorphosis, although worthy of careful consideration, cannot be regarded as an important factor in determining the relationships of insects, according to this writer. An ancestral group, it is contended, may include some forms which have "developed the tendency towards a metamorphosis, to a marked degree, while other representatives of the same ancestral group do not exhibit any marked indications of such a tendency." Plecoptera, Embiidae, Dermaptera, Coleoptera and their allies constitute the "plecopteroid superorder" and are regarded as the ancestral group from which the higher insects were derived. This group contains forms exhibiting well marked metamorphosis and some which do not. The higher

forms are divided into two super orders: (1) the "psocoid superorder" containing the Psocodae, Mallophaga, Anopleura (Pediculidae, s.b.), Hemiptera, Homoptera and their allies—a group in which few members exhibit traces of metamorphosis; and (2) the "neuropteroid superorder" comprising the Neuroptera, Hymenoptera, Mecoptera, Diptora, Siphonaptera, Trichoptera, Lepidoptera and their allies, all being predominantly holometabolous. Thus it is suggested that we might expect the coleopterous representatives of the ancestral group to be somewhat nearer the derived holometabolous group, while the remaining representatives of the ancestral group would be nearer the derived non-metabolous group. To account for the origin of metamorphosis among some of the ancestral forms, it is thought that there arose a tendency (by mutation, etc.) of the immature stages to differ from the adults, resulting eventually in stages which could enter an environment untenable by the adult. Such forms, favored by natural selection, would tend to persist and thus there would appear a "propensity towards the production of complete metamorphosis." Against the claim of Handlirsch that *cold* produced metamorphosis, Crampton argues that "insects in which the tendency toward metamorphosis was *already well developed*, were better equipped than their less fortunate fellows, to penetrate the less favorable regions of winter-frost, etc., and there establish themselves." No support is found in embryology or palaeontology for the view that larval stages represent "free-living embryos." Disagreement with any view that environment *causes* metamorphosis is expressed. The pupal stage is regarded as the "making over" period necessitated when immature and adult stages come to differ so markedly that a great change must be involved in the transition. Larvae stages are regarded by this author as having some phylogenetic significance and may yield valuable hints as to relationships. Whether primitive types of larvae represent ancestral conditions more nearly than adults do seems uncertain. In some cases it seems to be true but in other instances the larvae have become far more specialized than the adult, thus involving secondary characters.

PAUL S. WELCH

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University of Michigan

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TRANSACTIONS
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PROTOZOA OF THE DEVIL'S LAKE COMPLEX, NORTH DAKOTA

BY
CHARLES HOWARD EDMONDSON, PH.D.
University of Oregon[1]

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1. INTRODUCTION

Several reports previously issued[2] have described the physiographic and chemical characteristics of Devil's Lake situated in Ramsey County, North Dakota. In a recent paper Dr. R. T. Young,[3] of the University of North Dakota, has indicated something of the possibilities and limitations of the lake from a biological point of view, as well as the general scope of the work already accomplished in that direction. It will only be necessary, therefore, to set forth a few of the specific features of this water area which may have some bearing on the report to follow.

[1] The investigations included in this report were carried on at the State Biological Station of North Dakota.

[2] Biennial Report of the State Biological Station of North Dakota, 1911–12.

Pope, T. E. B. Devil's Lake, North Dakota, a study of physical and biological conditions, with a view to the acclimatization of fish. U. S. Bureau of Fisheries Document 634, 1908.

Simpson, H. E. The Physiography of the Devil's-Stump Lake Region, North Dakota. Sixth Biennial Report of the State Geological Survey of North Dakota, 1912.

Upham, W. The Glacial Lake Agassiz, Mon. 25, U. S. Geological Survey, 1895.

[3] Young, R. T. The Work of the North Dakota Biological Station at Devil's Lake. The Scientific Monthly, December, 1917.

The origin of Devil's Lake may be traced to glacial action and at an early period the united waters of this region must have covered a very large area. In 1883 the United States Geological Survey estimated the area of the lake at 125 square miles, and at that time it had a depth of 60 feet, while at the present time its area is about one-half and its maximum depth one-third that of thirty-seven years ago. By a gradual drying up of the lake and the establishment of certain roadways, it has been divided into four larger bodies of water and a number of smaller ones. These four divisions have been designated as the Western Section, Main Lake, East Lake and Lamoreau Bay. To the southwest of Lamoreau Bay is situated Stump Lake, in times past connected with the main body and included in the complex for purposes of this report. Within recent years Big and Little Mission Lakes have been entirely cut off from the main lake at its southeast border, and parallel with the southwest border of the main lake are a number of smaller lakes, which at one time were parts of the common body of water. The concentration of the water of these lakes approaches or exceeds that of the main lake. Locally they are designated as lakes "C," "N," and "O." A limited survey of these lakes was made in connection with the preparation of this report.

A general recession of the lake by evaporation has naturally resulted in an increased salinity which, although varying considerably with the seasons and localities, amounts to approximately 1% at the present time. The solids are, for the most part, sulphates of sodium and magnesium and sodium chloride. The following table represents the relative amounts of salts as determined by the United States Bureau of Chemistry from samples of water taken in June, 1906:[4]

	Parts per Million
Calcium bicarbonate	119.8
Magnesium bicarbonate	647.6
Magnesium carbonate	167.0
Magnesium sulphate	1,470.0
Sodium sulphate	4,758.9
Sodium chloride	1,354.0
Total	8,517.3

[4] Pope, T. E. B. Devil's Lake, North Dakota, a study of physical and biological conditions, with a view to the acclimatization of fish. Bureau of Fisheries Document 634, 1908.

The following analysis was made by the United States Bureau of Chemistry in 1907, from samples taken at Station 6, East Lake:[4]

	Parts per Million
Carbonic acid ion	154.9
Bicarbonic acid ion	555.6
Silica	44.0
Chlorin	1,122.0
Iron	16.4
Calcium	31.2
Magnesium	601.6
Sulphuric acid ion	6,254.4
Sodium	2,725.3
Potassium	250.0

The concentration of the water from East Lake was somewhat higher than that from any one of five other localities, the analyses of which were made at the same time.

In the shallower parts and about the borders of the lake the ditch grass, *Ruppia maritima*, forms luxuriant growths, while the greater portion of the bottom is covered with a thick layer of ooze. In 1901 Dr. Heath,[5] then of the Department of Chemistry of the University of North Dakota, made the following analysis of the ooze from the bottom of Creel Bay, an arm of the main lake:

Volatile (mostly organic matter)	28.80%
SiO_2	27.43%
Insoluble sulphates	12.78%
Fe_2O_3 and Al_2O_3	13.11%
Calcium	8.97%
Magnesium	0.47%
Manganese	0.026%

Moore ('17),[6] in concluding a list of algae from Devil's Lake, says: "Excluding the diatoms, of which there seems to be a considerable number of species, the algal flora of Devil's Lake can hardly be said to be recorded as a rich one." This investigator attributes the almost total absence of certain groups of algae to the high content of salts in the water, but, in general, he finds the algal flora typically a fresh water one and showing little or no effect of the concentration of water.

[5] From unpublished notes.

[6] Moore, G. T. Preliminary list of algae in Devil's Lake, North Dakota. Annals of the Missouri Botanical Garden 4; 293–303, November, 1917.

Biological studies of Devil's Lake made by the United States Bureau of Fisheries in 1908 indicate the presence of four vertebrate inhabitants of the lake, namely: a stickleback, *Eucalia inconstans;* a minnow, *Pimephales promelas;* the hellbender, *Crytobranchus alleghaniensis;* and the leopard frog, *Rana pipens.* Among the metazoan invertebrates reported are crustaceans, rotifers, nematodes, a flat worm, an arachnid and a number of species of insects. One may collect the shells of at least fifteen molluscs from the water line on the shore, but no living forms have been taken from the lake. Sponges, coelenterates, polyzoans and annelids are apparently entirely absent.

Investigations of the protozoan fauna of the Devil's Lake complex were undertaken as a part of the general biological survey of that water area. Although, in many respects, this fauna was found to be such as one might expect in a fresh water lake of similar depth, yet some very pronounced differences were disclosed. The almost total absence of shell-bearing rhizopods may possibly find its explanation in the chemical analysis of the water. *Arcella vulgaris* Ehrenberg, a very constant and usually abundant form in fresh water, was rarely observed and two species of *Difflugia*, which are among the most common protozoa in lakes where there is considerable ooze, were taken only in situations where the salinity of the water must have been materially reduced by the in-seepage of surface water. A species of *Euglypha* was taken in the overflow of the lake water from the fish tank. The only other shelled rhizopod observed was a single specimen of *Cyhoderia ampulla* Leidy, taken from the main lake.

The fact that the ooze at the bottom of the lake at times has been found to be entirely free from oxygen might also be a contributing factor to the scarcity of these usually common bottom-dwelling rhizopods of the shell-bearing type, although the presence of the larvae of a certain midge in this ooze as well as the work of Birge and Juday in Wisconsin,[7] where a considerable number of animals were found at the bottom of lakes in the absence of oxygen, would hardly seem to make this factor one of great importance.

Experiments of a preliminary character, recorded at the end of the taxonomic part of this report, indicate that certain protozoa having

[7] Birge and Juday, The Inland Waters of Wisconsin; Wisconsin Geological and Natural History Survey, 1911.

adjusted themselves to fresh water conditions are not, in all cases at least, readily adaptable to the waters of Devil's Lake.

The writer wishes to acknowledge his indebtedness to Dr. R. T. Young, Director of the State Biological Station of North Dakota, whose co-operation made this report possible, and to Mr. E. G. Moberg for his valuable assistance in collecting material.

2. Taxonomy

SUBPHYLUM SARCODINA
CLASS RHIZOPODA
SUBCLASS AMOEBAE
ORDER GYMMAMOEBIDA

Family Amoebidae

Genus Amoeba Ehrenberg, 1831

Amoeba proteus (Rösel).

Der kleine Proteus Rösel, Insecten Belustigung, 1755, tab. 101.

Amoeba proteus Leidy, Pr. Ac. Nat. Sc., 1878.

Occurrence.—Associated with *Ruppia* in Whipple Bay, Creel Bay, Minnewaukon Bay, Six-mile Bay, East Lake, and also taken from the east side of the main lake and from the overflow of lake water from the fish tank near the laboratory.

Amoeba radiosa Ehrenberg.

Amoeba radiosa Ehrenberg, Abh. Akad. Wiss., Berlin, 1830.

Occurrence.—Rarely observed. Taken with *Ruppia* from Minnewaukon Bay, also from Big Mission Lake.

Amoeba limax Dujardin.

Amoeba limax Dujardin, Histoire Naturelle des Zoophytes Infusoires, Paris, 1841.

Occurrence.—Associated with *Ruppia* and algae at the head of Creel Bay, Big Mission Lake (numerous), Little Mission Lake (numerous), and the east side of the main lake (numerous).

Amoeba verrucosa Ehrenberg.

Amoeba verrucosa Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—Observed but once, from material taken along the east side of Creel Bay.

Amoeba guttula Dujardin.

Amoeba guttula Dujardin, Histoire Naturelle des Zoophytes Infusoires, Paris, 1841.

Occurrence.—Taken from algae near Brannon's Island, from both ooze and floating algae in Creel Bay, from the east side of the main lake, and from sediment on rocks near the Station.

Amoeba striata Pènard.

Amoeba striata Pènard, Études sur les Rhizopodes d'eau douce. Mem. Soc. Phys. et Hist. Nat. Geneve, 1890.

Occurrence.—One specimen only observed in plant infusion from Stump Lake.

Amoeba vitraea (Hertwig and Lesser).

Dactylosphaerium vitraem Hertwig and Lesser, Ueber Rhizopoden und denselben nahestehende Organismen. Arch. Mikr. Anat. Vol. 10, Suppl., 1874.

Occurrence.—Taken from the east side of Creel Bay.

ORDER TESTACEA

Family Arcellidae

Genus Difflugia Leclerc, 1815

Difflugia pyriformis Perty.

Difflugia pyriformis Perty, Zur Kenntniss kleinster Lebensformen in der Schweiz, 1852.

Occurrence.—Only observed from Big Mission Lake in a location where fresh water seeps into the lake.

Difflugia constricta Ehrenberg.

Difflugia constricta Ehrenberg, Abh. Akad. Wiss. Berlin, 1841.

Occurrence.—Taken from Big Mission Lake in the same situation as the preceding species, and also from the head of Creel Bay near the entrance of a sewer.

Genus Arcella Ehrenberg, 1830

Arcella vulgaris Ehrenberg.

Arcella vulgaris Ehrenberg, Abh. Akad. Wiss. Berlin, 1830.

Occurrence.—Taken in ooze from the head of Creel Bay and from near the station, also from Big Mission Lake near the in-seepage of fresh water; abundant in the latter locality.

FAMILY EUGLYPHIDAE

Genus Cyphoderia Schlumberger, 1845

Cyphoderia ampulla (Ehrenberg).

Difflugia ampulla Ehrenberg, Bericht Preuss. Akad. Wiss., 1840.

Occurrence.—One specimen only has been observed. Taken from Whipple Bay among *Ruppia*.

Genus Euglypha Dujardin, 1841

Euglypha alveolata Dujardin.

Euglypha alveolata Dujardin, Histoire Naturelle des Zoophytes Infusoires, 1841.

Occurrence.—Taken from the overflow of lake water from the fish-tank near the Station. Observed but once.

SUBCLASS HELIOZOA
ORDER APHROTHORACIDA

Genus Actinophrys Ehrenberg, 1830

Actinophrys sol Ehrenberg.

Actinophrys sol Ehrenberg, Abh. Akad. Wiss., Berlin, 1830.

Occurrence.—Rarely observed, taken from among *Ruppia* in Minnewaukon Bay.

SUBPHYLUM MASTIGOPHORA
CLASS ZOOMASTIGOPHORA
SUBCLASS LISSOFLAGELLATA
ORDER MONADIDA

FAMILY RHIZOMASTIGIDAE

Genus Cercomonas Dujardin, 1841

Cercomonas sp. Figures 1–3, Plate XVIII.

Probably *Cercomonas longicauda* Dujardin. Very plastic with caudal filament often developed. Diameter, when spherical, 10μ

Occurrence.—Observed in infusions from Stump Lake only.

FAMILY HETEROMONADIDAE

Genus Monas Müller, 1786

Monas sp. Figures 4, 5, Plate XVIII.

Very plastic. Diameter, when spherical, 20μ. May represent *Monas fluida* Dujardin.

Occurrence.—In the ooze from Creel Bay.

Monas sp. Figure 8, Plate XVIII.

Length 9μ; body persistent in form, anterior region very granular. Corresponds in some degree to *Monas irregularis* Perty.

Occurrence.—In the ooze from Creel Bay. From a stale culture of *Ruppia*, Creel Bay.

Monas sp. Figure 7, Plate XVIII.

Body moderately plastic. Length, when extended, 15-18μ. Possibly same as figures 4 and 5.

Occurrence.—In the ooze from Creel Bay.

ORDER HETEROMASTIGIDA

FAMILY HETEROMITIDAE

Genus Heteromita Dujardin, 1841

Heteromita globosa (Stein).

Bodo globosus Stein, Der Organismus des Infusionthiere, Abth. 3, 1878.

Occurrence.—In dredged material from Creel Bay.

Heteromita sp. Figure 6, Plate XVIII.

But little of detail determined. Length 5μ. The form probably represents *Heteromita ovata* Dujardin.

Occurrence.—Taken from ooze on rocks near the Station.

ORDER POLYMASTIGIDA

FAMILY POLYMASTIGIDAE

Genus Trepomonas Dujardin, 1841

Trepomonas agilis Dujardin.

Trepomonas agilis Dujardin, Histoire Naturelle des Zoophytes Infusoires, 1841.

Occurrence.—Taken from Big Mission Lake, Whipple Bay, from the ooze of the main lake and from the east side of the main lake. Abundant in the latter locality.

ORDER EUGLENIDA

FAMILY EUGLENIDAE

Genus Euglena Ehrenberg, 1830

Euglena viridis Ehrenberg.

Euglena viridis Ehrenberg Abh. Akad. Wiss., Berlin, 1830.

Occurrence.—Observed from Minnewaukon Bay, Big Mission Lake, in the ooze from Creel Bay and from the east side of the main lake.

Euglena desus Ehrenberg.

Euglena desus Ehrenberg, Abh. Akad. Wiss., Berlin, 1830.

Occurrence.—Minnewaukon Bay, Six-mile Bay, near Brannon's Island, Big Mission Lake, Little Mission Lake, East Lake, and the ooze from the main lake.

Genus Phacus Dujardin, 1841

Phacus pyrum (Ehrenberg).

Euglena pyrum Ehrenberg, Abh. Akad. Wiss., Berlin, 1830.

Occurrence.—Minnewaukon Bay, Creel Bay, Big Mission Lake (numerous), and the east side of the main lake.

Genus Eutreptia Perty, 1852

Eutreptia viridis Perty.

Eutreptia viridis Perty, Zur Kenntniss kleinster Lebensformen in der Schweiz, 1852.

Occurrence.—From the surface among *Ruppia*, Big Mission Lake.

FAMILY ASTASIIDAE

Genus Astasia Ehrenberg, 1830

Astasia tricophora (Ehrenberg).

Trachelius tricophorus Ehrenberg, Abh. Akad. Wiss., Berlin, 1830.

Occurrence.—Among Ruppia from Whipple Bay, from Creel Bay, in the ooze from Big Mission Lake, and among algae near Brannon's Island.

FAMILY PERANEMIIDAE

Genus Petalomonas Stein, 1859

Petalomonas mediocanellata Stein.

Petalomonas mediocanellata Stein, Der Organismus der Infusionsthiere, 1878.

Occurrence.—Taken from the surface of Big Mission Lake and from the ooze of the main lake.

Petalomonas sp. Figure 10, Plate XVIII.

Has some resemblance to *Petalomonas ervilia* Stein. Conspicuous groove entire length of the body. Length 36μ.

Occurrence.—From the ooze of Creel Bay.

Genus Heteronema Dujardin, 1841

Heteronema acus (Ehrenberg).

Astasia acus Ehrenberg, Abh. Akad. Wiss., Berlin, 1830.

Occurrence.—From Six-mile Bay and from the ooze of Creel Bay.

Genus Anisonema Dujardin, 1841

Anisonema grande (acinus) (Ehrenberg).

Bodo grandis Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Anisonema acinus Dujardin, Histoire Naturelle des Zoophytes Infusoires, 1841.

Occurrence.—Among *Ruppia* and algae at the head of Creel Bay.

Genus Notosolenus Stokes, 1884

Notosolenus sp. Figure 9, Plate XVIII.

Length about 15μ.

Occurrence.—From Whipple Bay, Stump Lake and from the overflow of the fish-tank near the Station.

ORDER CHLOROFLAGELLIDA

FAMILY TETRAMITIDAE

Genus Tetraselmis Stein, 1878

Tetraselmis cordiformis (Carter).

Cryptoglena cordiformis Carter, Annals of Natural History 1858.

Occurrence.—Taken from Stump Lake only.

FAMILY POLYTOMIDAE

Genus Polytoma Ehrenberg, 1838

Polytoma uvella Ehrenberg.

Polytoma uvella Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—Found at the head and along the east side of Creel Bay.

FAMILY TRIMASTIGIDAE

Undetermined genus

Undetermined species. Figures 11, 12, Plate XVIII.

Description.—Body elongate, somewhat compressed, slightly plastic, attenuated posteriorly; surface marked longitudinally by several conspicuous ridges; flagella three in number arising from the anterior extremity, equal and equalling the body in length; nucleus and contractile vacuole unobserved. Length 20μ.

Occurrence.—Numerous among *Ruppia* from Creel Bay.

FAMILY CHLAMYDOMONADIDAE

Genus Chlamydomonas Ehrenberg, 1833

Chlamydomonas pulvisculus Ehrenberg.

Chlamydomonas pulvisculus Ehrenberg, Abh. Akad. Wiss., Berlin, 1833.

Occurrence.—Taken from the head of Creel Bay.

SUBCLASS DINOFLAGELLIDA
ORDER DINIFERIDA

FAMILY PERIDINIIDAE

Genus Glenodinium Ehrenberg, 1832

Glenodinium pulvisculus Ehrenberg.

Glenodinium pulvisculus Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—Taken from the surface and from the ooze at the bottom of Creel Bay.

SUBPHYLUM INFUSORIA
CLASS CILIATA
ORDER HOLOTRICHA

FAMILY ENCHELINIDAE

Genus Holophrya Ehrenberg, 1831

Holophrya ovum Ehrenberg.

Holophrya ovum Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—Among algae from Creel Bay.

Holophrya sp. Figure 13, Plate XVIII.

Resembling *Holophrya ovum* Ehrenberg but much smaller. Length 30-40μ.

Occurrence.—In the ooze from Creel Bay.

Genus Urotricha Claparède and Lachmann, 1858

Urotricha labiata, new species, Figure 14, Plate XVIII.

Description.—Body ovate, about twice as long as broad, equally rounded at both extremities. Cilia covering the entire body, arranged in longitudinal rows and vibrating independently. A very fine seta, nearly as long as the body, extending from the posterior extremity. Mouth anterior, subterminal, beneath a prominent, lobe-like lip. Nucleus central. Contractile vacuole posterior. Reproduction by transverse fission. Length of body about 30μ.

Occurrence.—Taken from numerous localities in Devil's Lake.

Genus Prorodon Ehrenberg, 1833

Prorodon teres Ehrenberg.

Prorodon teres Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—Among *Ruppia* and algae of Big Mission Lake and the main lake.

Prorodon edentatus Claparède and Lachmann.

Prorodon edentatus Claparède and Lachmann, Études sur les Infusoires et les Rhizopodes, 1858.

Occurrence.—Infusions of *Ruppia* from Big Mission Lake and Minnewaukon Bay.

Prorodon griseus Claparède and Lachmann.

Prorodon griseus Claparède and Lachmann, Études sur les Infusoires et les Rhizopodes, 1858.

Occurrence.—Taken from Stump Lake only.

Genus Enchelys Ehrenberg, 1838

Enchelys sp. Figure 15, Plate XVIII.

Length from 15-20μ.

Occurrence.—Ooze from the main lake and from the overflow of lake water from the fish-tank.

Genus Spathidium Dujardin, 1841

Spathidium spatula Dujardin.

Spathidium spatula Dujardin, Histoire Naturelle des Zoophytes Infusoires, 1841.

Occurrence.—Among algae from the head of Creel Bay.

Spathidium sp. Figure 16, Plate XVIII.

A very long, narrow and flattened form. Length 120μ.

Occurrence.—Taken from infusions from the head of Creel Bay.

Spathidium sp. Figure 17, Plate XVIII.

A much shorter form than the preceding, with a conspicuous collar about the oral extremity. Length 30μ.

Occurrence.—From the ooze of the main lake.

Undetermined Genus[8]

Undetermined species. Figures 1, 2, Plate XIX.

Description.—Body elongate, plastic, slightly compressed dorsoventrally, inflated posteriorly, narrow anteriorly, rounded at both extremities; cilia of uniform length arranged in longitudinal rows, covering the entire surface; aperture a narrow slit diagonally placed, sub-terminal; contractile vacuole posterior; nucleus concealed; endoplasm completely filled with green chloroplasts. Length 90μ.

Occurrence.—From the surface of the main lake and from among *Ruppia* and algae.

Genus Chaenia Dujardin, 1841

Chaenia teres Dujardin.

Chaenia teres Dujardin, Histoire Naturelle des Zoophytes Infusoires. 1841.

Occurrence.—Among algae from the head of Creel Bay.

Genus Mesodinium Stein, 1862

Mesodinium pulex (Claparède and Lachmann).

Halteria pulex Claparède and Lachmann, Études sur les Infusoires et les Rhizopodes, 1858.

Occurrence.—A common form on the surface and in the ooze of the main lake.

[8] The form is treated here with doubt as to its taxonomic position.

Genus Didinium Stein, 1859

Didinium nasutum (Müller).

Vorticella nasutum Müller, Animalcula Infusoria Fluviatilia et Marina, 1786.

Occurrence.—Among *Ruppia* from Minnewaukon Bay, Whipple Bay, and from the east side of the main lake.

Genus Lacrymaria Ehrenberg, 1830

Lacrymaria olor Ehrenberg.

Lacrymaria olor Ehrenberg, Abh. Akad. Wiss., Berlin, 1830.

Occurrence.—Among *Ruppia* in Creel Bay.

Lacrymaria truncata Stokes.

Lacrymaria truncata Stokes, Ann. and Mag. Nat. Hist., June, 1885.

Occurrence.—Among *Ruppia* from the north end of the main lake.

Lacrymaria cohnii Kent.

Lacrymaria cohnii Kent, A Manual of the Infusoria, 1881–1882.

Occurrence.—In an infusion from Stump Lake.

Lacrymaria lagenula Claparède and Lachmann.

Lacrymaria lagenula Claparède and Lachmann, Études sur les Infusoires et les Rhizopodes, 1858.

Occurrence.—In ooze from the main lake.

FAMILY TRACHELINIDAE

Genus Lionotus Wrzesniowski, 1870

Lionotus fasciola (Ehrenberg).

Amphileptus fasciola Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—Abundant in many parts of the main lake, also taken from Stump Lake and Big Mission Lake.

Lionotus sp. Figure 3, Plate XIX.

A very small species. Length about 40μ. Often seen in conjugation.

Occurrence.—Among algae from Creel Bay.

Genus Amphileptus Ehrenberg, 1830

Amphileptus meleagris (Ehrenberg).

Trachelius meleagris Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Amphileptus meleagris Claparède and Lachmann, Études sur les Infusoires et les Rhizopodes, 1858.

Occurrence.—Taken in Stump Lake and from algae at the head of Creel Bay.

FAMILY CHLAMYDODONTIDAE

Genus Nassula Ehrenberg, 1838

Nassula rubens (Perty).

Cyclogramma rubens Perty, Zur Kenntniss kleinster Lebensformen in der Schweiz, 1852.

Nassula rubens Claparède and Lachmann, Études sur les Infusoires et les Rhizopodes, 1858.

Occurrence.—From the overflow of lake water from the fish-tank near the Station.

Nasula ornata Ehrenberg.

Nasula ornata Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—Taken from Lake "N" only.

Genus Chilodon Ehrenberg, 1833

Chilodon cucullulus (Müller).

Colpoda cucullulus Müller, Animalcula Infusoria Fluviatilia et Marina, 1786.

Occurrence.—Infusions of algae from Creel Bay, Big Mission Lake, and Whipple Bay.

Chilodon caudatus Stokes.

Chilodon caudatus Stokes, Am. Jour. Sci. 29, April, 1885.

Occurrence.—Among *Ruppia* from Minnewaukon Bay.

Genus Aegyria Claparède and Lachmann, 1858

Aegyria pusilla (?) Claparède and Lachmann.

Aegyria pusilla Claparède and Lachmann, Études sur les Infusoires et les Rhizopodes, 1858.

Occurrence.—Among algae near the Station.

FAMILY CHILIFERIDAE

Genus Glaucoma Ehrenberg, 1830

Glaucoma scintillans Ehrenberg.

Glaucoma scintillans Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—In algae infusion from near Brannon's Island.

Glaucoma margaritaceum (Ehrenberg).

Cyclidium margaritaceum Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Cinetochilum margaritaceum Perty, Zur Kenntniss kleinster Lebensformen in der Schweiz, 1852.

Occurrence.—Very abundant. From the ooze of Creel Bay, the surface of Creel Bay, Stump Lake, and near Brannon's Island in the main lake.

Genus Leucophrys Ehrenberg, 1830

Leucophrys patula (Müller).

Trichoda patula Müller, Animalcula Infusoria Fluviatilia et Marina, 1786.

Occurrence.—One specimen only observed, from the east side of the main lake. A very typical specimen.

Genus Frontonia Ehrenberg, 1838

Frontonia leucas Ehrenberg.

Frontonia leucas Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—Taken from the east side of the main lake and from East Lake. Abundant in Six-mile Bay and Minnewaukon Bay.

Genus Loxocephalus Eberhard, 1868

Loxocephalus granulosus Kent.

Loxocephalus granulosus Kent, A Manual of the Infusoria, 1881–1882.

Occurrence.—Taken only in the ooze of Big Mission Lake near the in-seepage of fresh water.

Genus Uronema Dujardin, 1841

Uronema marinum Dujardin.

Uronema marinum Dujardin, Histoire Naturelle des Zoophytes Infusoires, 1841.

Occurrence.—One of the most common species in the lake. Abundant everywhere both at the surface and in the ooze.

Genus Colpidium Stein, 1868

Colpidium putrinum Stokes.

Colpidium putrinum Stokes, Ann. and Mag. Nat. Hist. Feb., 1886.

Occurrence.—From algae at the east side of Creel Bay.

Genus Tillina Gruber, 1879

Tillina saprophila Stokes.

Tillina saprophila Stokes, Am. Nat., Feb., 1884.

Occurrence.—Taken only in the overflow of lake water from the fish-tank near the station.

FAMILY PARAMAECIDAE

Genus Paramaecium Müller, 1786

Paramaecium trichium Stokes.

Paramaecium trichium Stokes, Am. Naturalist, 19, May, 1885.

Occurrence.—From near the mouth of a sewer at the head of Creel Bay, and from ooze near the rock pile in the main lake.

Paramaecium caudatum Ehrenberg.

Paramaecium caudatum Ehrenberg. Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—Taken from Big Mission Lake near the in-seepage of fresh water.

FAMILY PLEURONEMIDAE

Genus Cyclidium Ehrenberg, 1838

Cyclidium glaucoma Ehrenberg.

Cyclidium glaucoma Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—Abundant everywhere, at the surface and in the ooze in all parts of the lake.

Cyclidium litomesum Stokes.

Cyclidium litomesum Stokes, Am. Monthly Micro. Jour., 6, Dec. 1884.

Occurrence.—Numerous in infusions from the head of Creel Bay and in the ooze from the main lake.

Genus Pleuronema Dujardin, 1841

Pleuronema chrysalis (Ehrenberg).

Paramaecium chrysalis Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Pleuronema crassa Dujardin, Histoire Naturelle des Zoophytes Infusoires, 1841.

Occurrence.—Observed in infusions from Stump Lake only.

ORDER HETEROTRICHA

FAMILY PLAGIOTOMIIDAE

Genus Metopus Claparède and Lachmann, 1858

Metopus sigmoides (Müller).

Trichoda sigmoides Müller, Animalcula Infusoria Fluviatilia et Marina, 1786.

Occurrence.—Common in dredged material from Minnewaukon Bay, Creel Bay, and the main lake. Abundant in East Lake.

Genus Spirostomum Ehrenberg, 1835

Spirostomum ambiguum Ehrenberg.

Spirostomum ambiguum Ehrenberg, Abh. Akad. Wiss., Berlin, 1835.

Occurrence.—Observed in dredged material from Creel Bay.

FAMILY HALTERIDAE

Genus Halteria Dujardin, 1841

Halteria grandinella (Müller).

Trichoda grandinella Müller, Animalcula Infusoria Fluviatilia et Marina, 1786.

Halteria grandinella Dujardin, Histoire Naturelle des Zoophytes Infusoires, 1841.

Occurrence.—Common in infusions of *Ruppia* and algae from Whipple Bay and Creel Bay and in the ooze of the main lake.

ORDER HYPOTRICHA

FAMILY OXYTRICHIDAE

Genus Uroleptus[9] *Ehrenberg, 1831*

Uroleptus agilis Englemann.

Uroleptus agilis Englemann, Zeit. Wiss. Zool., Bd. 11, 1861.

Occurrence.—From the ooze of the main lake, also from Six-mile Bay.

Uroleptus rattulus (?) Stein.

Uroleptus rattulus Stein, Der Organismus der Infusionsthiere, 1859.

Occurrence.—Among *Ruppia* from Whipple Bay.

Genus Oxytricha[9] *Ehrenberg, 1830*

Oxytricha fallax Stein.

Oxytricha fallax Stein, Der Organismus der Infusionsthiere, 1859.

Occurrence.—Among algae from Creel Bay.

Oxytricha pellionella (Müller).

Trichoda pellionella Müller, Animalcula Infusoria Fluviatilia et Marina, 1786.

Oxytricha pellionella Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—Taken from *Ruppia* near the Station, Big Mission Lake, Whipple Bay, north end of Creel Bay, and the ooze from the fish-tank after being flooded by lake water.

Oxytricha parvistyla Stein.

Oxytricha parvistyla Stein, Der Organismus der Infusionsthiere, 1859.

Occurrence.—Among *Ruppia* near the Station.

Oxytricha bifaria Stokes.

Oxytricha bifaria Stokes, Ann. and Mag. Nat. Hist., Aug., 1887.

Occurrence.—Abundant in Creel Bay, also taken from Whipple Bay.

[9] Further study would, no doubt, result in the determination of other species of the genus than those listed.

Genus Histrio Sterki, 1878

Histrio erethysticus Stokes.

Histrio erethysticus Stokes, Proc. Am. Philos. Soc. 24; 126, 1887.

Occurrence.—Among *Ruppia* from near the Station.

Genus Stylonychia Ehrenberg, 1830

Stylonychia notophora Stokes.

Stylonychia notophora Stokes, Ann. and Mag. Nat. Hist. June, 1885.

Occurrence.—With algae from Creel Bay.

Genus Holosticha Wrzesniowski, 1877

Holosticha vernalis (?) Stokes.

Holosticha vernalis Stokes, Ann. and Mag. Nat. Hist., Aug., 1887.

A form bearing considerable resemblance to Stokes' species was occasionally observed. Length 140μ.

Occurrence.—Among *Ruppia* from the main lake.

Genus Pleurotricha Stein, 1859

Pleurotricha lanceolata (Ehrenberg).

Stylonychia lanceolata Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Pleurotricha lanceolata Stein, Der Organismus der Infusionsthiere, 1859.

Occurrence.—Taken at the head of Creel Bay.

Genus Tachysoma Stokes, 1887

Tachysoma parvistyla Stokes.

Tachysoma parvistyla Stokes, Ann. and Mag. Nat. Hist. Aug., 1887.

Occurrence.—Observed in infusions from Stump Lake only.

FAMILY EUPLOTIDAE

Genus Euplotes Ehrenberg, 1831

Euplotes charon (Müller).

Trichoda charon Müller, Animalcula Infusoria Fluviatilia et Marina, 1786.

Euplotes charon Ehrenberg, Die Infusionsthiere als Volkommene Organismen, 1838.

Occurrence.—Abundant among infusions of *Ruppia* and algae from many parts of the main lake, and also from East Lake.

Euplotes patella (Müller).

Kerona patella Müller, Animalcula Infusoria Fluviatilia et Marina, 1786.

Euplotes patella Ehrenberg, Die Infusionsthiere als Volkommene Organismen, 1838.

Occurrence.—Found in Stump Lake, Big Mission Lake, East Lake and in numerous localities in the main lake.

Genus Aspidisca Ehrenberg, 1830

Aspidisca costata (Dujardin).

Coccudina costata Dujardin, Histore Naturelle des Zoophytes Infusoires, 1841.

Occurrence.—Taken in Whipple Bay; numerous among *Ruppia* in Minnewaukon Bay and also on the east side of the main lake.

ORDER PERITRICHA

FAMILY VORTICELLIDAE

Genus Vorticella Linnaeus, 1767

Vorticella telescopica Kent.

Vorticella telescopica Kent, a Manual of the Infusoria, 1881–1882.

Occurrence.—Among *Ruppia* at the north end of the main lake.

Vorticella convallaria Linnaeus.

Vorticella convallaria Linnaeus, Systema Naturae, Ed. 12, 1767.

Occurrence.—Attached to diatoms in the main lake, also among *Ruppia* in Big Mission Lake.

Vorticella octavo Stokes.

Vorticella octavo Stokes, Ann. and Mag. Nat. Hist., June, 1885.

Occurrence.—Among *Ruppia* at the north end of the main lake.

Vorticella microstoma Ehrenberg.

Vorticella microstoma Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—Taken at the east side of the main lake.

Vorticella sp. Figure 4, Plate XIX.

A very common form, resembling *Vorticella rabdostyloides* Kellicott but is considerably smaller and the body is transversely striated. Length of stalk 12μ, with the diameter of the body nearly the same.

Occurrence.—Attached to floating diatoms.

Vorticella sp. Figure 5, Plate XIX.

A species with more elongate body than the preceding but also transversely striate. Length of body 28μ, stalk 68μ.

Occurrence.—Attached to floating diatoms.

Genus Gerda Claparède and Lachmann, 1858

Gerda annulata, new species. Figure 10, Plate XIX.

Description.—Body elongated, cylindrical, of nearly equal diameter throughout, curved when extended; surface finely striate transversely; a prominent annular ridge present usually about one-fourth the distance from the posterior extremity; peristome border revolute, disc slightly elevated; contractile vacuole conspicuous; nucleus not observed. Length of body, extended, 80μ.

Occurrence.—Among algae and *Ruppia* from the north end of the main lake.

Genus Epistylis Ehrenberg, 1830

Epistylis plicatilis Ehrenberg.

Epistylis plicatilis Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—From the east side of Creel Bay.

Epistylis branchiophila Perty.

Epistylis branchiophila Perty, Zur Kenntniss kleinster Lebensformen in der Schweiz, 1852.

Occurrence.—Among algae near the head of Creel Bay.

Genus Carchesium Ehrenberg, 1838

Carchesium epistylidis Claparède and Lachmann.

Carchesium epistylidis Claparède and Lachmann, Études sur les Infusoires et les Rhizopodes, 1858.

Occurrence.—Among algae from Creel Bay.

Genus Zoothamnium Ehrenberg, 1838

Zoothamnium alterans Claparède and Lachmann.

Zoothamnium alterans Claparède and Lachmann, Études sur les Infusoires et les Rhizopodes, 1858.

Occurrence.—Among *Ruppia* and algae from Stump Lake.

Zoothamnium sp. Figure 6, Plate XIX.

Stalk very stout, zooids smooth, usually 2-8 in a colony. Length of stalk 216μ, of zooid 64μ.

Occurrence.—From Stump Lake, East Lake, Creel Bay, Whipple Bay, and from the main lake. Attached to algae or *Ruppia*. A fairly common form.

Genus Vaginocola Lamarck, 1816

Vaginocola crystallina Ehrenberg.

Vaginocola crystallina Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—Numerous among algae from East Lake, also taken from Stump Lake and from the north end of the main lake.

Genus Cothurnia Ehrenberg, 1831

Cothurnia imberbis Ehrenberg.

Cothurnia imberbis Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—Commonly attached to floating diatoms, from dredged material and also among *Ruppia* in Creel Bay. Also taken from Stump Lake.

Cothurnia curva Stein.

Cothurnia curva Stein, Der Organismus der Infusionsthiere, 1859.

Occurrence.—Among *Ruppia* at the north end of the main lake.

CLASS SUCTORIA

FAMILY PODOPHRYIDAE

Genus Podophrya Ehrenberg, 1838

Podophrya libera Perty.

Podophrya libera Perty, Zur Kenntniss kleinster Lebensformen in der Schweiz, 1852.

Occurrence.—Numerous at east side of the main lake.

Podophrya sp. Figure 9, Plate XIX.

Bears some slight resemblance to *Podophrya cyclopum* Claparède and Lachmann. The lobulated border may have represented a reproductive phase or possibly was abnormal. Total height 60μ, stalk 20μ.

Occurrence.—Attached to algae from the main lake. Several specimens were observed by Dr. R. T. Young.

Genus Sphaerophrya Claparède and Lachmann, 1858

Sphaerophrya magna Maupas.

Sphaerophrya magna Maupas, Arch. de Zoologie Experimentale, tom 9, Nov., 1881.

Occurrence.—From Stump Lake and the east side of the main lake.

FAMILY ACINETIDAE

Genus Acineta Ehrenberg, 1838

Acineta sp. Figure 7, Plate XIX.

Body triangular in broad view, compressed; endoplasm very granular, nucleus concealed. Total height 50μ, stalk 20μ. This species resembles, in some degree, *Acineta lemnarum* Stein.

Occurrence.—From floating material in the main lake and also among algae from Stump Lake.

Acineta sp. Figure 8, Plate XIX.

Body oval, slightly broader distally, greatly compressed; endoplasm granular concealing the nucleus and contractile vacuole.

Total height 60-72μ, stalk about 15μ.

Occurrence.—Attached to algae from Stump Lake. Commonly feeding on *Uronema*.

3. EXPERIMENTS

Preliminary experiments in transferring protozoa from fresh water to the concentrated water of Devil's Lake and vice versa.

In order to test the reactions of certain protozoa taken from other sources to the more concentrated waters of Devil's Lake a series of simple experiments were carried out by which forms of protozoa common to fresh water were transferred directly into the more saline water of the lake.

Infusions from a small body of fresh water near the southern boundary of the main lake were prepared and certain protozoa which readily appear in cultures were used in the tests.

By placing a drop of the fresh water culture on one end of a microscopic slide and a drop of lake water near the middle of the slide and, with a needle, drawing out from each drop toward the other a narrow channel of water until the two met, the protozoa were conducted from the fresh water drop into that of the lake water. To eliminate possible influence of the fresh water a series of drops of lake water were used and the organisms rapidly transferred from one to the other until they reached a pure medium of lake water.

The waters from the two sources were kept at a uniform temperature and the effect of the change of environment thus brought about was carefully noted by the activity of the organisms.

In similar manner the transference of certain protozoa from lake water to fresh water was accomplished and the effect of such change observed as hereinafter noted.

A. Transference of protozoa from fresh water to lake water.

1. Paramaecium sp. A specimen of a species, probably *Paramaecium caudatum* Ehrenberg, commonly occurring in the fresh water was removed to the pure lake water with the following results: An immediate change occurred in the organism. The body became greatly compressed dorso-centrally with erratic movements at first which soon gave way to a more steady, forward movement with slow rotations on the long axis. A noticeable change also occurred in the contractile vacuoles. The normal rhythmic collapse of the vacuoles ceased after a few minutes and they became greatly dilated and distorted. After ten minutes of rotary movements the organism became quiet with the cilia of the periphera and the oral groove still active. Many non-contractile vacuoles filled the endoplasm. Death occurred at the end of twelve minutes.

A second specimen, after showing the same flattening of the body, moved in circles for six minutes then assumed the forward movement with rotations on the long axis. In eighteen minutes the organism became quiet with a highly vacuolated endoplasm and the cilia of the oral groove vibrating feebly. Death occurred in twenty-six minutes.

A third specimen after exhibiting similar physical and physiological changes came to complete rest in twenty-two minutes. Death resulted in twenty-five minutes.

A fourth specimen showed similar responses and died in fifteen minutes.

Seven specimens were then transferred at the same time. Six of these, after exhibiting similar responses as the preceding, were dead at the end of ten minutes. One, after reacting in like manner, died at the end of eighteen minutes.

2. Stylonychia sp. Several tests with a species of *Stylonychia* were carried out. Unusual responses were less quickly manifested by *Stylonychia* than *Paramaecium* when brought into contact with the lake water. Commonly after five or six minutes of normal movements a rapid whirling over and over of the body occurred gradually subsiding into complete rest. Death occurred in all specimens in from sixteen to thirty-two minutes.

Reactions of similar character were obtained from *Paramaecium* and *Stylonychia* by the introduction of small quantities of NaCL into the fresh water in which they were normally living.

3. Metopus sp. A short type of *Metopus*, common in fresh water, was transferred to the saline lake water. The most noticeable change was an almost immediate flattening of the body. Normal rotary movements continued for eight minutes when the organism came to rest with the cilia of the surface still more or less active. Death occurred at the end of fifteen minutes.

Numerous individuals of this species were used in successive experiments with reactions similar in each case. Death resulted in all specimens in from eleven to eighteen minutes.

B. Transference of protozoa from the concentrated lake water to fresh water.

1. Uroleptus sp. The form used was one of the elongated types. More than sixty specimens were used in the tests. With few exceptions but with considerable degree of variation, the following reactions were very evident: After a period of from ten to fifteen minutes contact with the fresh water, during which time more or less normal activities were maintained, the organisms came to rest with the cilia still in motion. The cell bodies became shortened and dilated, in

many instances assuming a spherical form. After enduring this state of depression for from ten to fifteen minutes the organisms showed signs of recovery. The bodies gradually assumed an elongated form and normal activities reappeared. Within a period of one hour and twenty-five minutes from the time the organisms were first introduced into the fresh water all, with the exception of a few which failed to survive the state of depression, had fully recovered and were responding in a normal manner.

Considerable variation in the effect of the change was noted. Of those surviving some were slightly affected and wholly recovered in forty-five minutes, some in sixty minutes, while others required the longer time noted above.

2. Euplotes patella (Müller). Numerous individuals of this species were transferred as in the preceding experiment. The effect in this case was an immediate one. As soon as contact was made with the fresh water the cell bodies became swollen and distorted, losing the longitudinal striations and all resemblance to normal individuals. During this state of depression the organisms were at rest with the cirri in feeble motion. After a period of fifteen minutes the cells began to resume movements although in a distorted condition. In fifteen minutes more the longitudinal striations reappeared and soon after normal responses were entirely restored.

3. Uronema marinum Dujardin. The transferrence of this species from the lake water to fresh water resulted in no apparent state of physical depression and no diminished or unusual responses to stimuli could be detected. The species is commonly recognized as both a marine and fresh water form.

4. Summary and Conclusions

Summary of the Groups of Protozoa Recorded

Sarcodina	13 species
Mastigophora	22 "
Infusoria	76 "
Total	111 species

Conclusions

1. The proportion of the number of species of the three groups of protozoa recognized in Devil's Lake corresponds favorably with the same in a typical fresh water lake.

2. A most noticeable feature of the study of this fauna is the apparent total absence of numerous forms universally found in fresh water. The dearth of shell-bearing rhizopods was mentioned in the introduction. Many common species of flagellates and ciliates were, at no time during the survey, observed in the concentrated waters of the lake.

3. The subdivisions of the classes of protozoa are fairly well represented in Devil's Lake. Two new species are described in the report but with the exception of the facts mentioned in the preceding paragraph, the protozoan fauna of Devil's Lake cannot be considered an unusual one.

4. Experiments of the interchange of protozoa between fresh water and the lake water seem to indicate that the organisms of the lake may adjust themselves to fresh water conditions with more readiness than can the forms accustomed to a fresh water environment accommodate themselves to the concentrated water of the lake.

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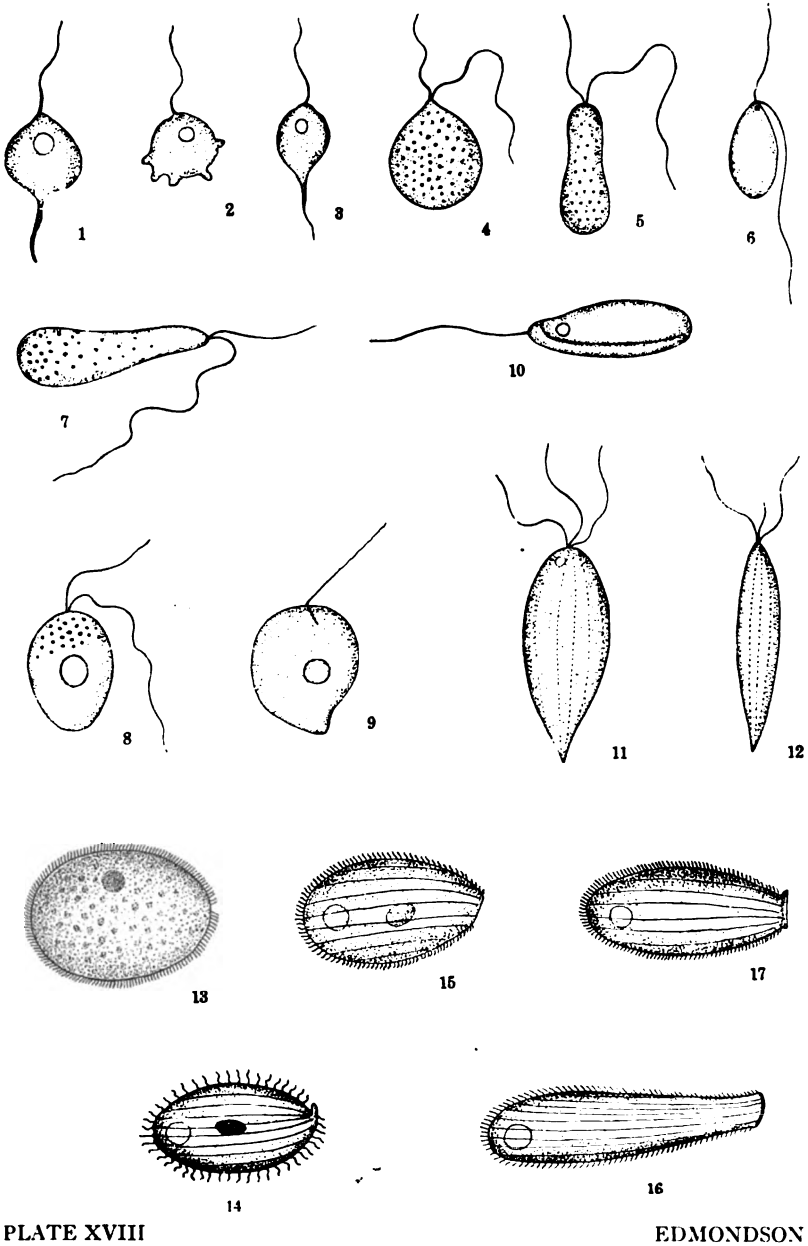

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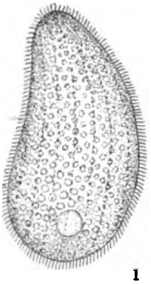
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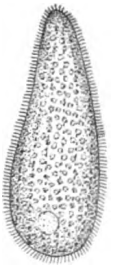
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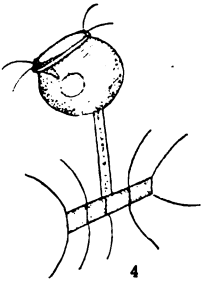
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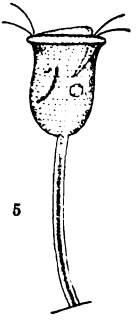
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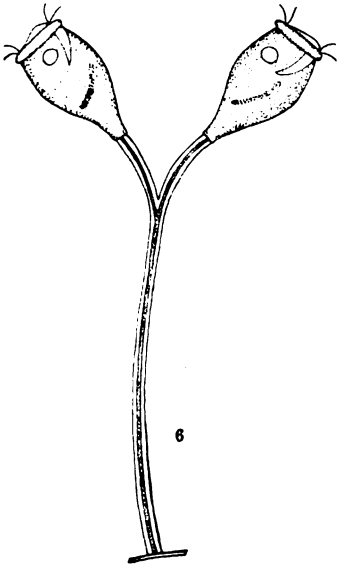
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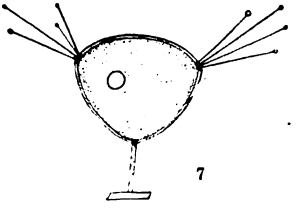
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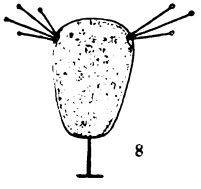
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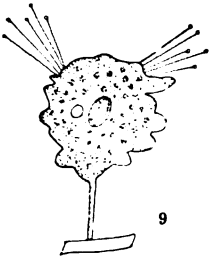
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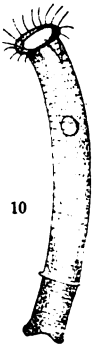
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PLATE XIX

EDMONDSON

AGE, GROWTH AND SCALE CHARACTERS OF THE MULLETS, *MUGIL CEPHALUS* AND *MUGIL CUREMA*

BY

ARTHUR PAUL JACOT

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INTRODUCTION

During the summers of 1915 and 1916 the writer was given the opportunity of studying the rate of growth and development of the mullet of the Atlantic coast of the United States. The collecting was done at and about Beaufort, N. C. Of the two species under consideration, the striped mullet (*Mugil cephalus*) has far the more economic importance. It ranges through all tropic and warm waters of the globe and has long been used as food. On our south Atlantic and Gulf coasts it has been sought so constantly and taken in such quantities that its numbers have noticeably decreased so that the supply continually falls short of the demand. For this reason the artificial propagation of this species is very desirable and it is towards this end that the present investigation has been made.

Differentiation of Species

Along the Atlantic coast from New England to Florida, two species of mullet may be encountered, *Mugil curema* and *Mugil cephalus*, the latter being much the more common. Commercially, no distinction is made although the fishermen seem to be aware of two species, calling the former the "silverside" or white mullet and the other the "jumping" or striped mullet. Other common names are locally used. When asked wherein they differ, the fishermen give a variety of more or less accurate answers, and generally end with some statement to the effect that the "silverside" is very seldom caught. A review of the fisheries literature on these species shows a lumping of the two, so that no accurate information concerning their respective habits can be secured.

Technically the silverside mullet (*M. curema*) differs from the jumping mullet (*M. cephalus*) in having more heavily scaled second dorsal and anal fins, nine rays in the anal fin in contrast to eight in *M. cephalus*, and 38 versus 42 scales in the lateral series and 12 versus 14 scales in transverse (diagonal) series. The field marks are the scalation of the anal and second dorsal fins and a lack of the longitudinal stripes of *M. cephalus*.

Because of the unreliability of single characters in species determination, and because of the possible difference in coloration of adult and young, a study of the variation of specific characters was necessary. Relative measurements in these two species are impracticable as the slight difference in ratios is repeatedly exceeded by individual variation. The amount of scalation on the second dorsal and anal fins is a fairly good character for both old and young fish, but is relative only. The *total* number of rays and spines in the anal fin is not constant. Specimens of *M. curema* with a total of 13 anal fin supports (rays and spines), the last of which may be bi- or tripartite, are not rare, while specimens with 11 supports are rare. *M. cephalus* has ten supports more often than twelve. Thus, though quite constant, this character cannot be wholly relied upon. This leaves the scalation of the two species to be considered.

The mullet is an unusually favorable subject for lepidology because of the relatively large scales and the presence of the lateral line groove (without the pore) which is found on nearly every scale, and which materially aids in the alignment of the scale rows. The

general results are that the number of scales in the lateral series varies considerably while the number in the transverse series is very constant. For instance, although *M. cephalus* normally has 42 scales in the lateral series, it often has 41 though as many as 44 and as few as 38 have been found. One specimen was found with 40 scales on the left side and 44 on the right side, two of the extra scale rows were situated between the base of the pectoral fin and that of the first dorsal, while the other two were below the second dorsal. On the other hand a stunted individual 185mm. long whose ratio of depth in length is 3 instead of 4, has 41 scales in lateral series and 15 in transverse series. Each scale, however, is much shorter along its cephalo-caudal axis than scales of normal fish. *M. curema* varies somewhat less. No variation was found in the number of lateral rows (number of scales in transverse series). Thus it would seem that the number of horizontal rows, as in the Ophidia (see Ruthven 1908), is a reliable species character, or at least more so than the number of transverse rows. Owing to the difficulty of counting the number of scales in transverse series of small specimens under a binocular microscope, due to the rotundity of the body and the consequent necessity of rotating the specimen while counting, this character was found impracticable for the determination of large numbers of young, and the scalation was, therefore, further studied. The results are represented in figure 1, which is reproduced from a fairly typical individual. In the absence of a lateral line row the median row on the caudal peduncle was chosen for counting the number of scales in lateral series. The last scale of this row is short and almost hidden by the

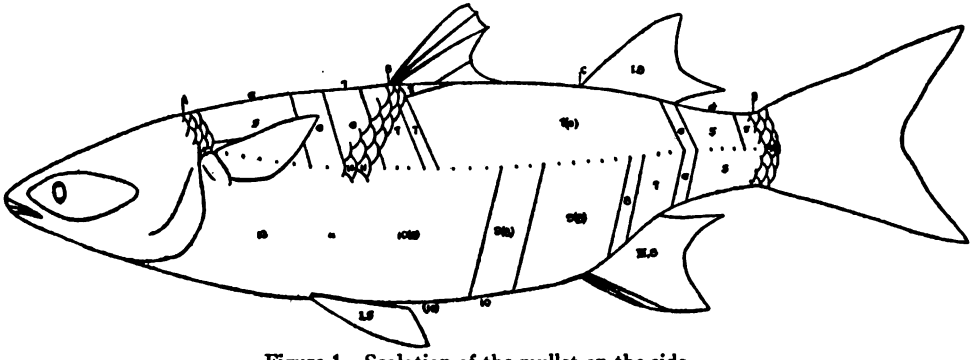

Figure 1. Scalation of the mullet on the side.

forty-first scale. The other scales of the last row become more evident dorsad[1] and ventrad. In the figure the numerals on the lateral median line designate the number of the transverse row, those on the body designate the number of scales in the transverse rows of that area except where a numeral appears above or below the body, in which case the numeral designates the total number, but as one of them is situated on the dorsal or ventral median line, that scale belongs as much to one side as to the other. All numerals include the lateral median line (except those upon it). The numerals in parentheses are the corresponding figures for *M. curema*, (where omitted they are not given). The reason for the diminution of the number of scales in the transverse rows caudad or cephalad is shown in figure 2

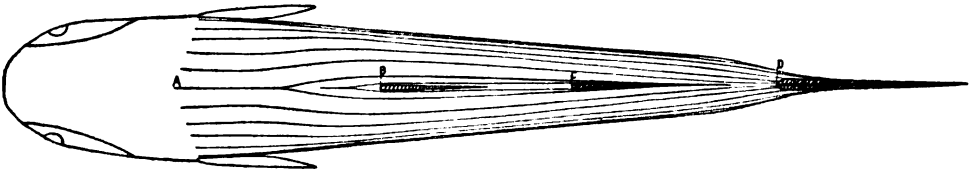

Figure 2. Scalation of the mullet on the back.

which is a dorsal aspect of figure 1 with the lateral line grooves connected by continuous lines. Thus when two lines run together, two scale rows become one row, and where a single line ends, a scale row becomes crowded out. A similar condition obtains on the venter. It therefore seems that reduction of scale rows occurs on the dorsal and ventral median lines—a condition very different from that in the Ophidia (Ruthven 1908). The exact location of the termination of a lateral row varies with the individual so that figure 2 is but individual and the area between *C* and *D* varies in appearance with each specimen. Likewise, there is variation in appearance between points *A* and *B* and the corresponding section on the venter. The area between points *B* and *C* down to, and including the venter, may be definitely relied upon as constant. The transition band on the ventral section (the area between 10(9) and 9(8)) is liable to shift caudad or cephalad a scale or two, but this should cause no confusion.

[1] The termination *-ad* as explained by Wilder and Gage (1882) signifies "towards," "in the direction of," etc.

From this analysis it should be evident that any variation in number of scales in the horizontal row will shift the *limits* of the various areas caudad or cephalad, depending on the individual, and this in turn means that only the middle area is unshifting. This middle area is also so broad that the desired flexibility in counting is given and the possibility of the complete loss of a row is greatly minimized.

A total of forty-four catches made between December 22 and September 4, during several years were plotted on co-ordinate paper using the abscissae for the length of the fish and the ordinates for the date of capture. Simple lines are used for *M. cephalus*, and railroad lines for *M. curema* and *harengus* (see fig. 3). The length of speci-

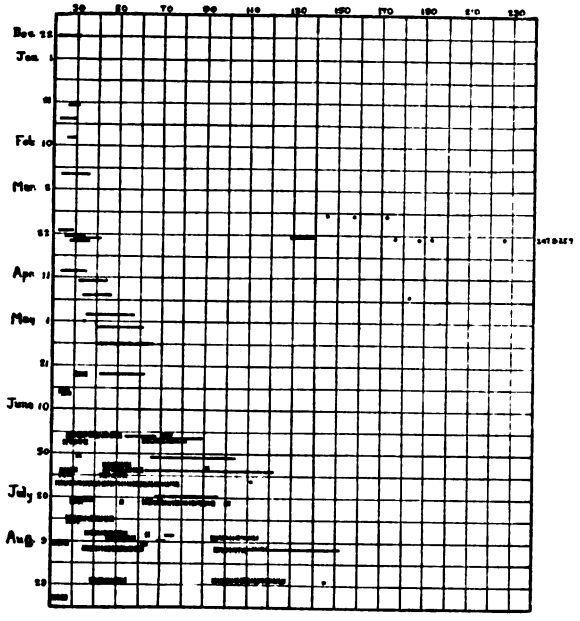

Figure 3. Record of non-linead mullets.
— = M. cephalus.
= M. curema.

mens herein given is the greatest possible length and all measurements unless otherwise stated are in millimeters.

MUGIL CEPHALUS LINNÉ

Determination of Young

Before anything can be done with the young of *M. cephalus*, it will be necessary to go back to *Myxus harengus* of Günther. In 1883 this species was established as the type of a new genus *Querimana* (Jordan) which differs from the genus *Mugil* in having the following characters—a serrated preorbital, thin lips, no adipose eyelid, stronger teeth and two instead of three anal spines. Bean (1903) has described the development of a third anal spine from the first ray. Further investigation has brought out the fact that the adult also has a serrated preorbital, as will later be described. The condition of the lips, teeth and adipose eyelid will, in the proper place, be shown to be but juvenile characteristics. Thus the genus *Querimana*, consisting of juvenile mullet, becomes a synonym of *Mugil*.

The specimens of *M. cephalus* ranging from 23mm. up to 40 or 50mm. were very carefully examined and were found to be juvenile *M. cephalus*, a heretofore undescribed *Querimana* (having the "Querimana" formula of A. II, 9; scales 42–14).

The specimens running into the *M. curema* group answer perfectly to the description given for *Q. harengus*. The description of *Q. harengus* (Jordan, 1896), gives it thirty-eight scales in the lateral series, twelve in transverse series and an anal fin formula of II, 10. Adulting (changing to adult condition) this fin formula according to the evidence given by Bean (1903) we have A. III, 9. This agrees with *M. curema*. The development of *Q. harengus* further shows it to be the young of *M. curema*, as will be shown below and not a distinct species.

Development of Young

The juvenile stage of *M. cephalus* begins with individuals as small as 23mm. Their first appearance is in the form of well developed fish without the slightest larval appearance. As already described by various writers, they form compact schools, swimming near the surface of the water. They may be found in deep water, or more often in water but a few inches in depth. The time of the year during which they are to be found may best be seen by consulting figure 3. They might easily be mistaken for the young of *M. curema*, because

of the similar coloration. Collections made from December to March consist of slim silvery individuals of small size. There seems to be very little growth during this time. The sides are devoid of pigment, being sharply defined from the dark brownish-green back. In later March and early April this dark dorsal band is extended down the sides by the gradual appearance of pigment cells. By mid-April these pigment cells have so increased as to merge the dark back into the silvery venter. With this advance in color, the fish rapidly increases in length and the abdomen is bulged by the developing intestine. Besides these external evidences of a turning point in the life history of the species, the growing parts of the fish show this change, though some more strongly than others. In the following the juvenile characteristics of this species through the development of the fish to its adult form, this turning point has been noted and the reason sought. The lengths of the fish as given below are used for the purpose of correlating the development of the various parts with with the fish as a whole, and are of typical specimens. The silvery (or juvenile) stage is found in specimens from 23 to 32mm. in length, while those from 30 to 35mm. are somewhat difficult to distinguish as silvery or dusky because of the merging of the two forms at this size.

The *preorbital* in the juvenile stage has some 10 or 12 points, teeth or serrations, of fair size. As the fish grows these points become more and more numerous, less slender and less distinct. In older fish they become blunt and stocky until in a large individual (502mm.) there were 53 teeth on the margin, crowded so as to place about four to a millimeter.

The *adipose eyelid* shows no marked acceleration in rate of growth at the end of the silvery stage. It is entirely lacking in the smallest specimens, but by the time the fish has reached a length of 28mm., with the aid of a high power binocular microscope, a slight translucent growth can be detected just anterior to the eye. In describing the state of transparency of the eyelid, it must be remembered that only alcoholic specimens are being described, in life the adipose eyelid being perfectly transparent at any age. For the sake of convenience the eyelid has been divided into three parts: (a) the ring, which is situated about the rim of the orbit, (b) the anterior lobe, and (c) the posterior lobe. When the fish is about 30mm. long, the anterior lobe

having slightly thickened, has become semi-translucent and has stretched backward over the eye. At 32mm. length, the anterior lobe has further thickened and become slightly more opaque, stretching farther back over the eye and merging into the ring which has just become visible. By the time the fish is 36mm. long, the anterior lobe has become opaque, while the ring, which has very slightly stretched posteriorly, has become semi-translucent. From this stage, the gradual development of the growth can be easily followed without the aid of the microscope. At 39 mm. the posterior lobe has become quite definite while the anterior has thickened and become more nearly opaque. On 42mm. specimens, the anterior lobe is visible to the unaided eye. The ring has assumed an opaque cast at its inner edge and stretched out over the rim of the nearest scales. The posterior lobe has, by now, spread out over the preopercle but is still translucent. The growth of the orbital ring is now very slow, its chief expansion being inward over the eye. In a specimen 47mm. in length the posterior lobe has become so opaque as to become visible to the unaided eye. Its lateral growth takes it not farther up or down than the outer diameter of the ring, while its chief growth is posteriorly over the preoperculum. The anterior lobe grows no farther forward, having reached a point just anterior to the nostril, but it slowly grows out over the eye. At 54mm., the more rapid growth of the lobes has caused them to overrun the ring anteriorly and posteriorly, so that the ring now assumes an elliptical shape, the long axis of the ellipse being vertical. Sixty millimeter specimens show this ellipse more strongly developed, the posterior lobe much lengthened posteriorly and the whole eyelid in its typical form, so that it is now only a matter of slow growth before the adipose eyelid has assumed its maximum development in the full-grown adult. Thus it is seen that there is no break between the juvenile stage and the adult.

The *thin lips* given as a characteristic of the genus *Querimana* do not appear disproportionally thin for so small a specimen. If there is any relative thickening of the lips beyond normal development, it is so gradual as to be imperceptible.

That *the teeth* are slightly stronger in the juvenile young than in the adult cannot be considered a generic difference in itself, as it may well be due to a retrograde modification due to change in food habit; as there seems to be grounds to suspect is the case.

The development of *the third anal spine* from a ray was described by Bean (1903) but deserves further comment. The ray is simple and has about four articulations. At the close of the juvenile stage

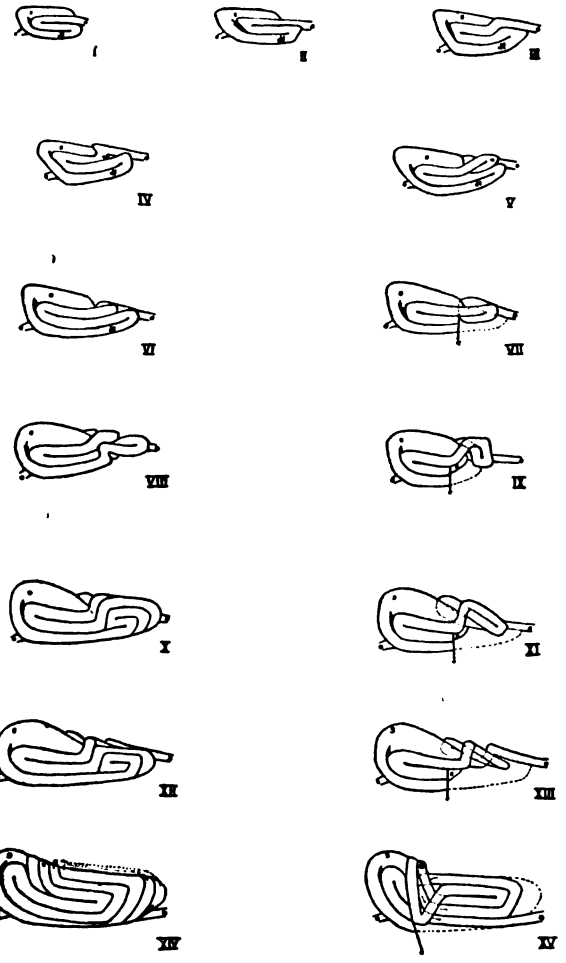

Figure 4. Development and convolutions of the intestine of *Mugil cephalus* when from 23 mm. to 40 mm. in total length. s=stomach; e=esophagus; a=anus; c=line of cut of duodenal loop, see p. 208. At figure v the fish is 32 mm. long and transforming into the dusky stage.

this ray ceases growing with the same rapidity as the true rays and becomes heavier basally, continuing to become relatively heavier and stiffer, until it is about one-third of the space between the tips of the second spine and the first ray, longer than the second spine spine (Fig. 1). This relative length is maintained throughout life. As this spine continues growing and thickening, the articulations become obliterated until lost so that in adults the third spine is basally as heavy as the second and quite equal to it as a spine. This development should be of much interest to the morphologist and systematist.

The *reproductive organs* are so rudimentary as to be invisible throughout any part of the season or at any point in the juvenile stage. This might be sufficient reason in itself for discarding the genus *Querimana*.

The *development of the intestine* gives further evidence of the relations of the two forms. Owing to the difficulty of describing this development a series of outline sketches (Fig. 4) have been prepared, illustrating, by a lateral aspect, each successive change.

The most simple form (fig. 4, I) consists of a duodenal loop and a "straight-away" to the anus. When the figure V stage is reached the fish is passing into the dark or dusky stage, the kink *n* having lengthened into a loop whose lower member has twisted upward and over its upper one to form a loop. At the next figure (VI) the spleen appears as a yellowish body, 25mm. in diameter, and from then on becomes a factor in influencing the convolutions of the intestine. At the figure VIII stage the duodenal loop makes a kink which soon becomes a loop and thus destroys the duodenal loop in its typical form. Each odd figure from VII to XV shows the convolutions on the further or inside by the cutting away of the duodenal loop or its modification at line c. Beyond the stage shown in figures XIV-XV the convolutions become so intricate that their study would surpass the scope of this paper, the length of the fish at this time averaging about 40mm. Thus, in the lengthening of the intestine, there is a marked acceleration in the rate of growth at the time when the fish is about 32mm. long, i.e., when the fish is passing into the young or dusky stage.

Besides this development in length and complexity of the intestine proper, the whole abdominal cavity is eloquent of the change exter-

nally noticeable. To appreciate this change, it is necessary to begin with the earliest individuals. All December specimens examined had their entire viscera and the walls of the abdomen colored orange, while the peritoneum in many cases was grayish with dark spots, otherwise it was of a semi-translucent blackish color. The length of the intestine was at times half that of the individual itself, though generally about three-quarters its length. In January, the coloring of the intestine was the same as for the previous month with the exception of a few individuals in which it was yellowish while the peritoneum averaged darker, and the walls of the abdomen a little lighter. The length of the intestine showed a slight increase over specimens of corresponding lengths of the previous month. In February the viscera were yellowish to pale, a very few individuals having traces of vegetable matter in the intestine. The peritoneum showed no special change, while the walls of the abdomen were pale. The intestine, on an average, had increased in length to a slight extent, but in no cases equaled the length of the individual. In late March, quite a few specimens had entirely lost their internal orange or yellow color and the intestine had traces of dark matter. The peritoneum was black and the flesh about the viscera had assumed the more natural dark coloration. These specimens showed marked increase in length of intestine, it being considerably longer than the individual. These fish were passing into the dusky stage. The majority of specimens, however, were much like those of the previous month. The viscera of April specimens are rarely orange or yellow tinged, the great majority having the intestine more or less filled with dark matter. The length of the intestine had also correspondingly increased. These fish were well into the dusky stage, their intestine appearing as represented in VII to XV of figure 4. It was from these slowly developing individuals that material for figure 4 was taken. From this time on the growth of the fish is very rapid in comparison with that of the previous month. Thus the increased length of the intestine can be directly correlated with its own color.

An explanation for the change in visceral coloration described in the preceding paragraph was sought by examination of stomach contents. The silvery-sided individuals (juvenile fish) showed an almost exclusive diet of crustacea, mainly copepods, and as alcohol almost invariably turns this form of life a salmon red, the coloration of the

viscera is accounted for. The intestine as well as the stomach were filled with this food, the latter not yet having reached the gizzard development. In the most immature individuals the stomach's form was that of a simple sack. The stomach contents of the dusky stage consisted, roughly, of 40% sand and mineral matter and 60% vegetable and animal matter. This latter consisted of 50% diatoms, 35% algae and other soft vegetable matter and 15% animal miscellaneous. This seems to be the usual ration of the fish during the remainder of its life, it being known as a mud feeder (See also Linton 1913).

From the above, two things should be apparent, namely (a) that the first form (the juvenile or silvery stage) develops into the second (the dusky stage), (b) that the juvenile stage is one of slow growth and development which is more rapid after the fish has changed diet, (made evident by the change in the color of the viscera). Because the intestine, the stomach and the whole fish acquire an acceleration in rate of growth and development at the time of change of diet, we conclude that this period of change is due to change of diet.

A study of *the development of the scale* along with the development of the individual is essential to the correct understanding and interpretation of the adult scale. The simplest form procurable is found in the juvenile or silvery-sided individuals (figs. 8–9). Text figure 5 represents one of these scales (in its natural position) divided into areas using Masterman's (1913) method. Rather than deal with the axes, greater convenience is found in using the areas formed by these axes as designated in figure 5. The terms "dorsal" and "ventral" have not been used as no need was found for this differentiation of sides. In the scale work here presented the scales were taken from the lateral median line on the two rows originating at the latero-anterior edge of the first dorsal fin (scales 10 and 11 of figure 1), except in the very young, where this was done as nearly as possible. For convenience, the appearance of the scale is described by means of a formula in which *a*, *l* and *p* stand for the anterior, lateral and posterior areas respectively, while the number following each of these refers to the number of circuli in that area. Thus, the formula for the scale of figure 5 would be a. 11; l.0; p. 22. The two lateral areas generally differ in number of circuli when these are present; the average has then been taken. In the more advanced stages of growth the number of

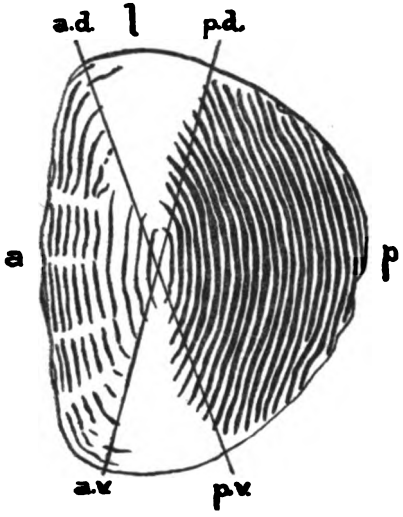

Figure 5. Advanced silvery stage scale, x45 divided into areas. a. v. = antero ventral axis; p. v. = postero-ventral axis; a. d. = antero dorsal axis; p. d. = postero-dorsal axis; l. = lateral areas; a. = anterior area or basal end; p. = posterior area or apical end. Formula = a. 11, l. o, p. 22.

circuli on the lateral area was computed by finding the average from two or three scales (taken from the place above mentioned from one or both sides of the fish). This does not affect the general result as whatever variation is shown in these four scales is about as great as the difference between the scales taken from corresponding places of two different fish of the same size from the same school. In other words, it was found that the average number of lateral circuli of scales 10 and 11 from fish number one was more constant than the average number of lateral circuli of scales 10 and 10 from fish number one and two (both being of the same size and from the same school). With this, it must be remembered that the number of circuli does not represent the number of "days" of growth, but that they testify to the approximate *development* of the individual. The formula, then, is useful in conveying a fairly good idea of the size and amount of development of the fish from which it was taken.

The smallest fish have a scale already well developed, a 23mm. specimen having a circuli formula a. 7; l. 0; p. 15 (fig. 8). This being

the type of scale showing the least growth of any procurable, it is inferred that the greater portion of this scale was formed at the spawning grounds. Notice on this scale, (a) that the circuli on the posterior area are much closer together than those of the anterior area, (b) that the lateral areas are without circuli and (c) that the circuli nearest the center are farther spaced than those further out. As the scale enlarges, more circuli form on its anterior and posterior edges, until the scale has reached a maximum development of a. 11; l. 0; p. 22 on a fish of 32mm. length. This type of scale may be found on any silvery-sided individual, i.e., during the months of December, January, February, many in March, and a few in April. The addition of circuli during this season is very slow, so that the scale in three months' time, shows no more advance than illustrated in figure 9. Very often the scale shows less development than this before the juvenile stage comes to a close. From this point, the method of growth of the scale completely changes. Figure 10 shows a scale whose formula at the silvery-side stage was a. 10; l. 0 p. 20. A little later two *closely spaced* anterior circuli were added, and, while this was going on, the tenth anterior circulus stretched back along the outer rim of the scale, thus forming a lateral circulus, so that the formula of the complete scale has become a. 10+2; l.0+1; p. 20, and the posterior edge of the scale shows a narrow border without circuli. In the next figure (11) three anterior circuli have been added to the juvenile scale, two of which have become lateral; the posterior border is quite wide but without definite circuli. Note the shallow depression just posterior to the center. This is the beginning of what is, in some fish, known as the lateral line groove, and in this paper will be referred to by this term. Figure 12 has two more anterior circuli, an additional lateral circulus, a broken or fragmentary posterior circulus with suggestions of a second, and a larger lateral line groove. Note the fine reticulations at the anterior edge of the posterior circuli. The fish from which the scale of figure 11 was taken had a length of 38mm. The circulation is still further advanced giving the formula a. 9+10; l.0+4 or 5; p. 19+1(or 2). The lateral line groove almost obliterates the first few posterior circuli and the reticulation or veining is more extensive and better developed. This series should clearly show the way the scale changes its habit of sculpture (habit of growth of the configuration of the surface). Figure 14 shows a scale from a 45mm. individual and

gives the effect of this new development. The juvenile scale is seen to be completely encircled by the later more rapid addition of circuli which *tend* to be continuous. Thus, the scale is of the cycloid type. Note, (a) that the anterior circuli are almost twice as numerous as the lateral, their ends terminating near the anterior axes, (b) that the anterior circuli of the outer series curve in the opposite direction to those of the juvenile scale, thus making a definite demarkation between the two scales, (c) that in the outer series the closely spaced circuli are anterior while they are posterior in the juvenile scale, and vice versa with the widely spaced circuli, so that in this respect the habit of sculpture is reversed. This change, although taking place at the same time as a change in diet, and occurring during the months of March and April, is not due to seasonal or dietal change, for the scale of *M. curema* passes through the same change at a different season (during the summer) and unaccompanied by a change of food. It is therefore inferred that this change is due to some previous change in habit of growth of the scale, i.e., the change is phylogenic.

During this development the juvenile scale which is designated by various authors as the nuclear area, nucleus,[2] centrum, initial field, etc., occasionally passes through a process of deterioration of surface face sculpture. This begins with the veining just anterior to the posterior circuli (figs. 11–14) spreading farther and farther until the lateral areas are covered (figs. 17–19). When the lateral areas are fairly well filled in, the posterior circuli are gradually replaced by the veining so that the veined area is linear or ovate in shape and not the shape of the scale. A process giving a similar aspect has been described and accounted for by Dahl (1911, p. 11–13).

The addition of circula continues more or less regularly for a longer or shorter time according to the individual. Figure 15 illustrates a scale taken from a 60-70mm. specimen, and serves to show the nature of growth of the apical or posterior circuli. Note the way in which the posterior circuli are bending out toward the apex of the scale. With the bending out of these circuli the scale grows more rapidly at the apex and on this posterior lobe narrow, pointed, posteriorly directed cteni gradually rise from the surface. These cteni are firm and strong, much longer than wide, slightly bent to give

[2] It seems preferable to reserve the term "nucleus" for the structural center of the scale as used by Cockerell (1913).

more rigidity, and sharply pointed (figs. 20, 25, 27). These cteni continue to form row after row, the scale taking on the appearance of the one illustrated by figure 20. This scale (removed from a fish taken on the 23rd of August) contains all the characteristics of the species although the individual was but 145mm. long and not yet one year old. The lateral line groove has extended backward to the posterior margin of the juvenile scale and forward as a narrower channel to its anterior margin and to the posterior end of one of the basal radii. This linear shape is that assumed by the lateral line in the adult. In figure 22, although the scale shows nearly the same amount of growth, the cteni have not as yet begun to form. Before the further development of the scale is noted, it will be necessary to review what is known of the migration of this fish.

Migration

The earliest reliable information we have concerning the migration of the mullet is a note left by Dr. Yarrow (Smith 1907) on the fish in the Beaufort region in 1871. The substance of this note relative to migration is that small-sized individuals appear in May, and that in later August fish commence to school preparatory to migration. He says:

> The schools appear to come from the northward through Albermarle, Pamlico, and Core sounds, gradually working their way southward. Their departure through the various inlets seems to depend upon a favorable state of the wind, which should be from the northward, for it has been noticed frequently that when the wind hauled, the schools of mullet already without the harbor have suddenly turned, re-entering the inlet, and pursued their course southward through Bogue Sound.

A few years later the U. S. Commission of Fish and Fisheries sent out Mr. Ravenel (1887) to find out what he could about the mullet. The method pursued was to visit various fishing centers and consult the fishermen. The only reliable information we need note is that at Beaufort three "runs" were noted as follows:

small mullet	4–5 inches	June–Aug. 30.
fat	"	Sept.-Oct. 10.
roe	"	Oct. 10–Nov. 15.

The same year the Commission issued its comprehensive work on the fishery industries in which there are two papers on the mullet. The first one (Goode 1887) treating of the natural history will not

be considered as it is based almost entirely on hearsay but on the second (Earll 1887) which is much more comprehensive and reliable. From under its caption "movements" the following general notes have been extracted:

. . . Small sized individuals are scattered about on the feeding grounds in the grassy bays and marshes bordering the coast. Here they remain till late in July, when they proceed to the deeper channels of the larger bays, where they gather in schools of small size. Little is known of the whereabouts of the large mullet at this season. Later the migrations begin, the fish of medium size moving southward. Their places are soon filled by large fish. . . . These (roe mullet) remain until the first cold storm occurs, when they start for the south, moving rapidly along the outer shore, or through the inland passage. These fish are followed by smaller individuals known as "frost mullet," which remain through the greater part of the winter. The movement seems to be general along the entire coast, all fish along the Atlantic seaboard being reported as traveling southward, while those rounding Florida Keys continue their coastwise migrations, gradually working northward and westward towards the Texas line. No return movement is reported at any season along the Atlantic. . . .

In N. J. waters the mullet make their appearance in schools about the first of September, gradually working southward and entirely disappearing by the last of October. The same is true for the coast between Cape May and Cape Henry, including the waters of Chesapeake Bay.

The small fish are seen in June on the N. C. coast, these gradually increasing in numbers until the first of August, when the schools have attained considerable size, but thus far no tendency to migration is noticeable. A little later a southern movement begins, and school after school passes, the size of the individuals constantly increasing till the first of September when the old or roe mullet arrive. . . If the weather continues pleasant they remain along the shores until the eggs have become well developed before moving southward, but at the approach of the first cold storm they are off and other smaller individuals follow in their wake, so that by the first of January the greater part have disappeared. Comparatively few are seen from that date until the following June, though scattering ones may be taken at any time.

At Wilmington [N. C.] small mullet are occasionally taken at any season, though they are abundant from June to September only, and large ones are seen only in the fall. As at Beaufort, the migration begins about the middle of August. The first schools are composed of fish of medium size. . . . By the first of September these have entirely disappeared, and their places have been taken by the "fat mullet." These are very abundant for several weeks, the roe mullet arriving about the middle of October, before they have entirely disappeared. "Frost" or "inch" [the distance between the eyes] mullet, as they are sometimes called, follow in large, compact schools, the last disappearing about the middle of December. Smaller fish, called "winter-mullet," are abundant till spring. . . .

At Charleston the run is somewhat similar to that at Wilmington.

In East Florida, especially the St. John's River, fish of all sizes may be seen at any time. . . .

In the Gulf of Mexico it is claimed that the mullet are even more abundant than along our Atlantic coast. . . . They are never entirely absent, though, as on the Atlantic coast, they are much more abundant in the fall than at any other season. . . .

From the evidence at hand it is clear that the mullet fisheries for different parts of West Florida continue from the middle of August to the first of January, though the height of the season, for most localities, is in October and November. Farther west the fish seem less inclined to migrate, remaining more constantly in any given locality, and on the Texas coast it is said that there is no special time of abundance, but that mullet are equally plentiful at any season.

Notes on Wood's Hole (Smith 1897) state that *M. cephalus* is "Found from September to end of October, going in large schools about October 1." For the same region Sumner (1911) reports *M. cephalus* as "Present from July to December; most common in the fall." In summary, Bean (1903) states that about New York the earliest appearance of *M. cephalus* is in August when they are few, that in September they are found in the New York markets and that "the great schools were absent till October."

The two most striking facts brought out by this literature are those of a fall migration and the almost complete absence of large mullet on our coast during the later winter, spring and early summer. This migration seems to begin at the northern extremity of the range of the species and extends southward with the migrating fish. The migration seems to be orderly, deliberate, and in series, each series being made up of a certain age group, almost the whole coast load of mullet slipping around the peninsula of Florida and along the gulf coast before all have scattered through the more torrid water which is the real home of the mullet. Thus, there can be no question about a definite fall migration down the Atlantic coast to warm water. Another thing to be noticed and borne in mind is that the migration is slow and leisurely, taking at least three months, so that it would seem that each individual had time to live at its leisure on the way south. Finally, notice should be taken of the lack of any noticeable northward migration. Thus nothing is known of the fish from the time it reaches the gulf until it reappears in late summer. There can be no doubt that the fish does not return north during the winter, but that it is living in southern waters where it can feed unrestrain-

edly. After the winter therefore, in spring or early summer, this species must return north. For a possible record of this period of the life history of the fish, the scale may again be studied.

At the time that the young are from 40 to 60mm. long or about the beginning of May, individuals of another age-group, as small as 120mm. in length and up, make their appearance. These individuals keep increasing in size and numbers throughout the summer so that by the end of August they are very common and range from 220–370mm. in length. Their scales are all characterized by the single "line" or break in the continuity of the circuli typically illustrated in figure 25. The fish from which this scale was removed was taken on July 2 (1915) and had a total length of 218mm. Notice (a) the deterioration of the sculpture in the center, (b) the ctenoid area and the position of the sharpest and the most worn teeth, (c) the unpored lateral line groove, (d) the continuity of the "line" from the lateral area posteriorly to and into the ctenoid area, and anteriorly across the anterior area, (e) that the "line" is formed (1) laterally by the termination of the circuli in exactly the same way as they are terminated at the outer edge of the scale, and (2) anteriorly by the termination of the circuli in exactly the same way as they terminate at the anterior edge of the scale, (f) that this "line" is the so-called "winter-line" and (g) that the circuli within the "line" are all equally spaced. With the last three points (d, e, f) in mind as well as the fact that this is a south wintering fish, let us consult the scales of fish which remain in cold northern waters during the winter. Good illustrations of such scales have been published by Gilbert (1913), Mastermann (1913), Lea (1913), Nilsson (1914) and Hjort (1914) of the salmon, herring, mackerel and cod respectively. In the scales of the salmon and cod, close examination will reveal that the winter area is formed by the crowding together of the circuli (the circuli of the cod are broken into dashes). The mullet scale is entirely lacking in the crowding of circuli, testifying to undimished feeding during the winter. The herring and mackerel scales, due to non-concentric circuli on the older section of the scale show an entirely different type of "winter line." In this case it is formed by the pinching out of the circuli. Thus they cannot be used to compare with the mullet. Now, since the mullet is not affected by winter conditions and does not show the typical winter crowding of the circuli, another cause

must be sought for the break in the sequence of the circuli which does occur. As already pointed out, this break is exactly similar to the break caused by cessation of life. The break is sudden and complete. We advance the hypothesis that this line is caused by a spring migration differing from the fall migration in being made (typically) by a continuous run and not by a slow gradual shifting as in the fall. Various types of these "lines" or linea[3] may be encountered. Figure 25 illustrates its more typical and usual appearance, i.e., when the linea is similar to the periphery. An occasional type of linea consists of a straight but wide space between some two lateral circuli. In one scale examined practically all the pre-migratory lateral circuli had slightly shifted laterally and continued posteriorly as post-migratory circuli. This may have been due to a migration of such a nature that growth was retarded, not entirely stopped. Figure 24, if closely examined, will show two closely spaced lineae, the outermost being the most distinct. Such a form is occasional and may be due to a second migration several weeks after the first, the fish going still further north. Thus, the actual number of lineae cannot be absolutely relied upon for the age of the individual Furthermore, one cannot consider every linea a migration line as any cessation in feeding or growth for any reason whatever, might cause the interruption and renewed growth of the scale necessary to form a linea. Therefore, though the actual number of linea is not always reliable for the determination of the number of seasons which the individual has passed through, the linea may be relied upon for age determination when properly understood. Before this can be done, however, the development of the scale of the species must be studied along with the development and life history of that species.

The above mentioned hypothesis seems to be further substantiated when one notices that specimens from 129 to 257mm. long (clearly of a second age-component (fig. 3) having as many as 70 lateral circuli outside the juvenile scale) were taken in March. The lateral circuli of the scales of these individuals were all evenly spaced

[3] From the Latin *linea*, -ae, f; using the term in its more figurative application. I introduce this new term to specifically label the definite feature of the scale typically illustrated in figure 23 and explained above, restricting the terms peronidia, annuli, winter band, annular ring, etc., to the area of circuli between the lineae or between the first linea and the juvenile scale.

and no linea of any kind could be detected, yet they were passing through or had just passed through the winter, evidently at or in the general vicinity of Beaufort. Thus, all mullet do not leave our coast, those here in winter having probably come from much farther up the coast. Furthermore, no fish with a single linea were taken before late April which could at all be considered of this second age group (or younger). The fish scale from which figure 23 was taken was removed from an individual taken on the 28th of April. The linea is some three circuli from the margin of the scale, thus setting the date of migration during the earlier part of April. Other specimens taken during the spring have the following number of post-linea circuli:

May 2—2 and 4
May 4—4
May 11—7
May 12—0, 3, 6, 6, 7, 7, 7, 10
May 25—3 and 10

Allowing an average accretion of five circuli per month, this data gives early April as the norm of migration. That it is general and fairly definite is brought out by figure 6, which is the record of the

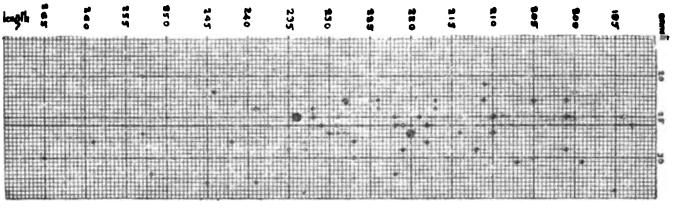

Figure 6. Record of Postmigratory annuli of Jumping Mullet caught July 10, 1915.

number of post-lineal circuli of a catch made on the tenth of July of one linead mullet. The fish being all taken on the same date, any variation of the date of migration should be shown by the number of circuli. The abscissae give the number of circuli and the ordinates the length of the specimens; the points of greatest magnitude designate three specimens recorded at that point, etc. Although there is a variation of 14 circuli, or nearly three months, it is not necessarily all due to difference in date of migration, for individuals vary in rapid-

ity of accretion of circuli, i.e., in a given time one individual may acquire x circuli while another would acquire x+3 or 4. However great or small a variation in time there may be, figure 6 clearly shows that there is a definite time of migration which, as has already been shown, takes place in earlier April and normally consists of a single continuous run from southern feeding ground to more northern waters. That the fish migrate in deep water off the coast seems evident from the fact that the fishermen are unaware of such a movement and that the fish is practically neglected by them until the fall migration.

Second Age-Group

The arrival of the jumping mullet in April marks the beginning of its second season on our Atlantic coast; its age ranges from 14 to 17 months and its size from 120 to 200mm. (5 to 8 inches) (see figs. 3

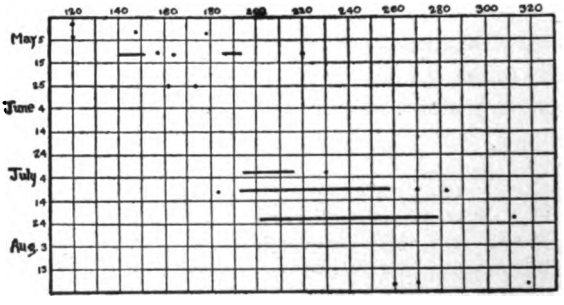

Figure 7. Record of one linead mullet.

and 7). "The individuals are scattered about on the feeding grounds in the grassy bays and marshes bordering the coast" (Earll 1887). They can be found in small numbers over any mud bottom, mud flats, etc., where vegetable plankton is abundant. Specimens may be secured at any time during the spring and summer but they are so scattered as to make fishing for mullet alone an expensive proposition. Living thus they grow to a length of from 225 to 325mm. by mid-August. Their flesh is very soft and oily, hence their name "fat mullet." By this time they have begun to gather into schools of ever increasing size, the social instinct becomes dominant as the reproductive organs rapidly develop. By late September the southward

migration has begun and as the fish move down the coast and the roe ripens, they spawn. Out of a batch of ten roe mullet purchased at the Beaufort market on October 9, 20 and 25, four had but a single linea as follows:

Length of fish 410,	Scale formula 1.68+50[4]
414	1.63+45
426	1.57+57
431	1.69+57

The remaining six each had two migration lines giving the following scale formulas:

Length of fish 426,	Scale formula 1.42+60+28
440	1.51+44+21
454	1.43+45+24
473	1.40+50+28
483	1.47+55+24
493	1.60+46+23

The small number of circuli acquired during the third year indicates that the rapid growth of the fish had been partially checked by having attained sexual maturity—as is usually the case. If the series examined was typical, and every effort was made to make a very general choice, this would mean that the jumping mullet generally attains maturity and spawns for the first time in its second year. That this is not always the case is evident from the scales of a male 392mm. long with testes 34mm. long and 7mm. wide, taken on July 12 (1915). The average number of lateral circuli for two or three scales reads 1.38+72+8, which means that it was spawned late in the season, that it probably migrated north early the first year, late in the second and in its third year was maturing early. No other fish was taken with reproductive organs so far advanced, so early in the year; the time of the year for organs of that size normally being in mid August.

Adults

According to scale evidence, the majority of jumping mullet breed for the first time during their second year. At this time they average

[4] As it is unnecessary to mention the number of circuli in the anterior area or in the juvenile scale, the formula for older fish need include only the number of circuli in the lateral area using a + sign for the linea.

less than a foot and a half, and constitute the great bulk of the mullet fishery. The largest mullet that has come under our observation is a 502mm. (20 inch) roe mullet whose scale (fig. 26), shows it to have been in its fifth year. Some individuals are reputed to attain a length of two and a half feet and a weight of ten pounds. What may be said concerning the average adult mullet applies equally well to the larger individuals. Those which return in the spring pass the time in the marshes, mud flats, and mud bottoms of the wide shallow estuaries, sounds, etc., so characteristic of our sunken and inundated Atlantic slope, at least as far north as Cape Cod. The colder temperature and rugged coast extending from Maine northward forms a highly efficient barrier for such a highly specialized fish as the mullet. In its spring and summer feeding grounds it can thrive secure from man for it is so scattered as to make seining unprofitable and it is in a practically inaccessible locality due (a) to the soft muddy bottom in which it seeks cover and in which man sinks so as to make seining impossible, (b) to the reeds and grasses over which the lead line will continually rise and allow the fish to run under—not to mention those which clear the floats with three to eight feet to spare, and (c) to the inaccessibility of the locality to power boats. Such is the choice feeding ground of the mullet, and such is the locality from which this fish returns to deeper water, fat and full, to enjoy a more gregarious and social life. As the schools increase in size and the temperature of the water lowers, their reproductive organs having developed, they move slowly down the coast *en masse* both outside and inside the Banks, spawning as necessity demands. At Beaufort roe mullet are rare in September, common in October, abundant in late October and early November, and rare in December; they are caught both inside and outside the banks, though (according to the fishermen) never with the eggs running (prime ripe); while spent mullet are found wherever mullet are to be found. Some of the fishermen attribute this lack of "running" roe mullet to their going out to sea to spawn while others claim that they spawn in fresh water because the young are found there (although they are equally abundant all the way out to well beyond the shore line). Thus nothing is known of the spawning grounds of this species and therefore of its eggs or larvae, the earliest stage known being the already well developed young described at the beginning of this paper.

MUGIL CUREMA CUV. & VAL.

Young

The young of the white mullet, as above shown, is the so-called *Querimana harengus*, and is undoubtedly found as far north as Wood's Hole. At Beaufort they have not been recorded earlier than May 25th, but there is reason to believe that they could be found even as early as late April. In habitat and habit they are similar to *M. cephalus*.

The *development* of this species is, in general, like the former, but without a definite silvery stage and with a constant rate of development of the various parts and of the individual. The smallest specimens normally procurable are 20 to 21mm. long and as much developed as are 23mm. specimens of *M. cephalus*. At this least size the alimentary canal contains no trace of the crustacean diet so characteristic of the other species, their stomachs being filled with the dark mud matter on which they continue to feed. Aside from this difference the two species are similar in their juvenile characteristics, i.e., they have cyclid scales, no adipose eyelid, and but two anal fin spines.

The *development of the scale* though mainly similar to that of the striped mullet is interestingly different. The juvenile scale differs from that of *M. cephalus* in a tendency toward one pair less of basal radii and in tending to have lateral circuli connecting anterior and posterior circuli (figs. 16–19). The lowest formula found was a 10, 1.0, p. 14, thus being more advanced than corresponding *M. cephalus*. As these juvenile fish acquire their adult characters the habit of sculpture of the basal area of the scale changes in the same way as does that of *M. cephalus*. The development of the apical portion of the scale, on the other hand, is strikingly different. In *M. cephalus* the lateral circuli generally extend backward following the contour of the juvenile scale until they meet and thus form about it a close fitting frame. This is so foreign to *M. curema* that it only rarely occurs and then only with the first circulus. The second one in extending backward tends to diverge from the first, the third from the second though possibly less, and so on (figs. 16–19). This occasionally occurs in *M. cephalus* scales (fig. 15) but only with a few circuli. The typical lateral circulation for *M. curema* scales is this

divergent type but without the close-fitting lateral circulus, the very first one forming an acute angle and terminating very briefly at the edge of the juvenile scale or continuing through it as an apical circulus. Meanwhile each apical circulus has done one of two things, it has entirely stopped growing or it has continued to grow. If all apical circuli cease growing at the same time another apical circulus may form about them as above described (figs. 16–19) and thus very definitely mark off the juvenile scale as in the other species, but, unlike it, this new circulus is close to the juvenile scale and immediately followed by others so that the apical circuli are much more closely spaced than in the jumping mullet. (Compare figs. 16–19 with figs. 12–15). If all the apical circuli of the juvenile scale continue to grow in full strength and unchanged direction (of very rare occurrence) the apical boundary of the juvenile scale is undiscernible. Although these circuli will continue to extend across the transition line between the juvenile and young scale, until they meet lateral circuli or reach an equivalent distance, they generally become thin at the transition line, or, in rare cases, become obsolete at that point, (figs. 16, 17). Accompanying this weakness of growth the circuli will often become curved or more widely or irregularly spaced at the transition line, so that the boundaries of the juvenile scale are plainly discernible. The first few apical circuli of the juvenile scale never run beyond it, extending only to the line of the posterior axes where they occasionally turn and become lateral circuli. In the great majority of juvenile scales all apical circuli do not pursue the same course, so that the scales present an enormous amount of variation on the transition line (figs. 16–19). For this reason it is very rare when the juvenile scale is not set off from the remainder of the scale posteriorly, while it is always discernible anteriorly. When apical circuli meet lateral circuli they do so at an acute angle thereby forming a type of circulation quite characteristic of the scale of this species (figs. 17–19, 21). Figure 16 shows such an angle just formed, another about to form, and another some distance from forming. Thus, although there is not so striking a transition in the scale of *M. curema* as in *M. cephalus*, yet there is a change so marked as to be unexplainable. It is certain that this change in the scale sculpture is not due to migration for all stages of the change, and scales some time before the change would take place,

are procurable as long as juvenile fish are obtainable, and further, the change is not merely a seeming cessation of growth for a short period, but a complete change in *sculpture habit;* nor is it due to change in diet for the intestine contents of the fish before and after the change, in the scale, are alike. Thus again the change seems to be recapitulatory or phylogenic. A factor in the destruction of the central sculpture, and more so than in the other species, is the spreading of the lateral line groove (figs. 17, 18).

After a various number of apical circuli have been formed (generally more than in *M. cephalus*) a break appears at the apical center in which cteni are formed (figs. 18, 19, 21). These cteni are added and develop much as in *M. cephalus*, but have an entirely different appearance. Instead of being narrow, slightly curved, keeled, and sharply pointed as in *M. cephalus*, the cteni of this species are wide and flat with a very inconspicuous keel at the apical end (figs. 27, 28). Moreover, the cteni in *M. curema* practically all appear in a well defined projecting band while in *M. cephalus* they gradually merge back into the old worn stubs of former teeth called by Cockerell (1913) "apical marginal elements" (herein, for brevity, called ctenobasii), and do not project as a well-defined band beyond the normal outline of the scale except in very advanced scales (fig. 26). In figure 27 notice how the ctenobasii seem in places to be broken up circuli and in others worn down cteni, as though the cteni were modifications of the circuli. In *M. curema* (fig. 28) the transition is not so gradual, the fringe of cteni seeming quite segregated from the remainder of the scale. The ctenobasii, however, are present in even greater numbers than in the other species and although they do not seem to be worn down cteni they occupy an area once covered by them (figs. 18, 19, 21). They must therefore be considered deteriorated cteni and noted as another difference between the two species. The cteni are added row after row along with the circuli throughout the summer until the fish have reached a maximum size of 230mm. in September when they migrate south. Figure 21 is from a scale of a 121mm. fish taken on the 23rd of August, and shows all the characteristics of the scale of this species. Compared with figure 20 (the corresponding scale of the other species) the radii are seen to be fewer in number. This is constantly the case. Both these scales having been taken from the same position on the fish's body, this difference is a real specific

difference. The lateral circuli are also more closely spaced in *M. curema* than in *M. cephalus* in scales of equal size. This does not mean that one species accrues circuli at a greater rate than the other.

Migration

In the fall the scattered individuals and small schools gather over the sandy bottoms in schools of ever increasing size, much as do the other species, and each school in its turn migrates leisurely south. During the winter this mullet is very rarely if ever found in the Beaufort region but with the approach of summer an occasional individual may be taken. It is, however, so uncommon in its second season or older, that the fishermen consider it a matter of curiosity or note when one is caught. Several specimens about eight inches long were taken on the 27th of June. From the scale formula of an individual 184mm. long (1.67+26) there seems to be little doubt that this fish migrated in the early spring. Three other scales from fish bearing no data show a similar linea, but situated farther from the edge of the scale.

Adults

Due to the scarcity of this species at Beaufort no true adults were procured so that practically nothing is known concerning their habits. From figure 3 it is evident that the spawning period must be rather protracted and, if an estimate of the time can be made from the dates when the smallest fish are procurable the season would be (conservatively) from mid-April to mid-August, the height of the season probably being in May.

Summary

Mugil cephalus Linné

1. To the synonymy of the genus *Mugil* should be added *Querimana.*

2. To the synonymy of the species *M. curema* should be added *Q. harengus*, its juvenile young.

3. *M. cephalus* spawns in October and November (September to December).

4. The juvenile young pass the winter without much growth.

5. In the spring the juvenile change diet and grow very rapidly until fall when they school and migrate south not to return until spring.

6. In the spring, the young, at that time from five to eight inches long, return north by a (typically) continuous run.

7. By the second fall the fish have reached a length of a foot or more and attained maturity.

8. In October and November these two-year-old fish migrate south spawning as they go.

9. Jumping mullet may attain an age of five or six years, spawning each year after maturity.

Mugil curema Cuv. & Val.

1. *M. curema* spawns in May and June (April to August).

2. The young are abundant in the bays and estuaries of our Atlantic coast and develop rapidly.

3. In the fall the young school and migrate south.

4. After their first year, white mullet are but seldom caught north of Florida.

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EXPLANATION OF PLATES

PLATE XX

Fig. 8. Juvenile scale from smallest fish normally obtainable (23 mm. fish), x 45.

Fig. 9. Juvenile scale with maximum amount of development (32 mm. fish), x 45.

Figs. 10–14. Juvenile scale being enclosed by the more advanced type of scale (29 mm.—45 mm. fish), x 45.

PLATE XXI

Fig. 15. Scale of a 60-70 mm. mullett with cteni first forming, x 45.

Figs. 16–17. Juvenile scale being enclosed by the more advanced type of scale, x 45.

Figs. 18–19. Development of cteni on scale of the white mullett, x 45.

PLATE XXII

Fig. 20. Typical scale of advanced first season jumping mullett, x 25.

Fig. 21. Typical scale of advanced first season 121 mm. white mullet taken August 23, x 21.

PLATE XXIII

Fig. 22. Scale of a 117 mm. mullet with unusual amount of circulation, x 30.

Fig. 23. Scale of 120 mm. jumping mullet taken April 28 with linea very near margin, x 25.

Fig. 24. Scale of jumping mullet, with a double linea, x 30.

PLATE XXIV

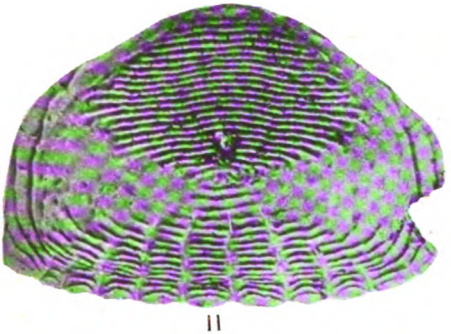
Fig. 25. Typical scale of second season jumping mullet. x 30.

PLATE XXV

Fig. 26. Scale of a five year jumping mullet 502 mm. (20 inches) long. x 13. Development of scale of *M. curema*.

PLATE XXVI

Figs. 27–28. Ctenoid area of scales of adult *M. cephalus* and *M. curema*, highly magnified.



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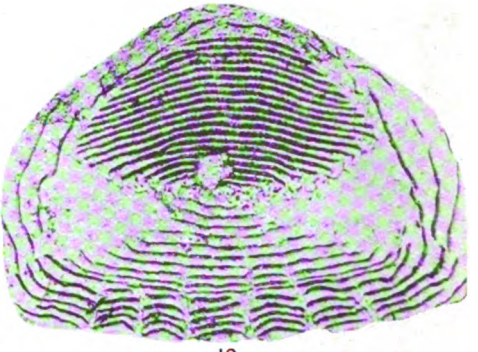

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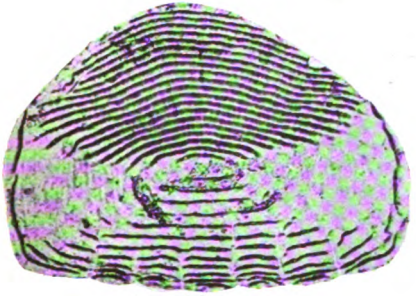

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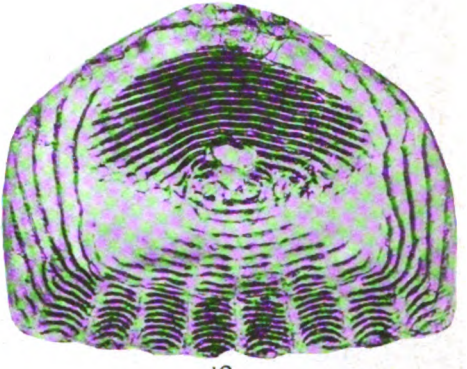

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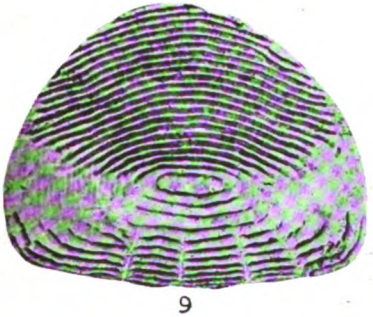

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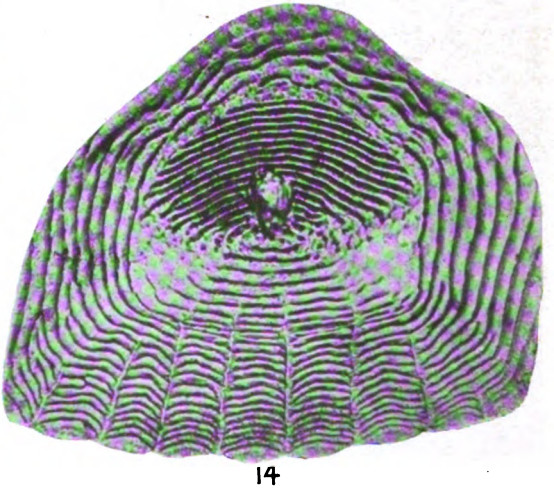

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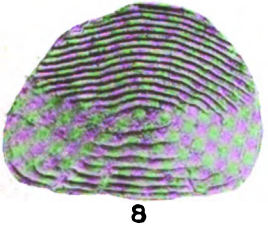

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PLATE XX

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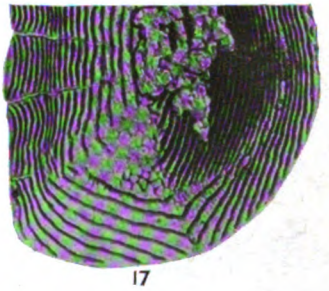

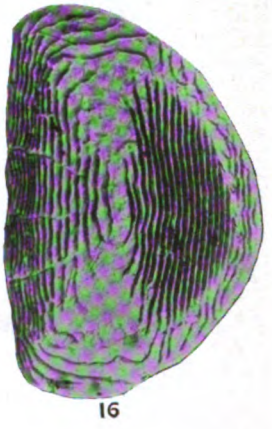

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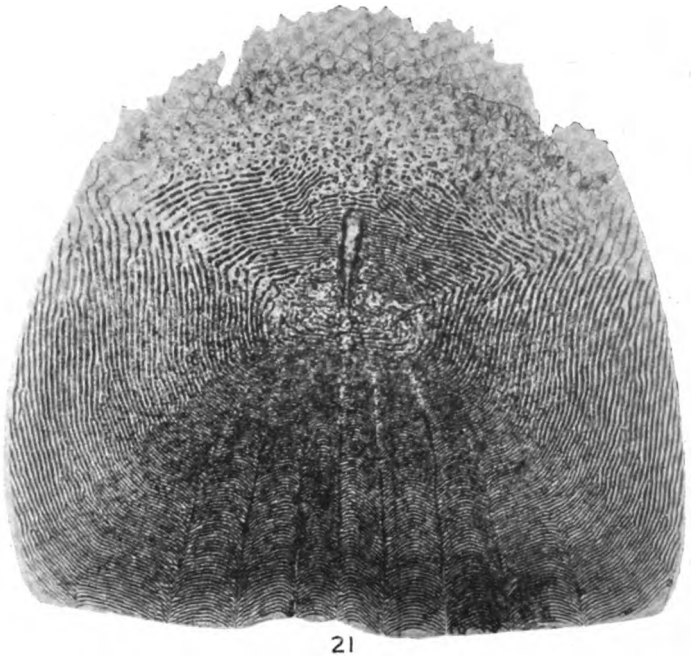

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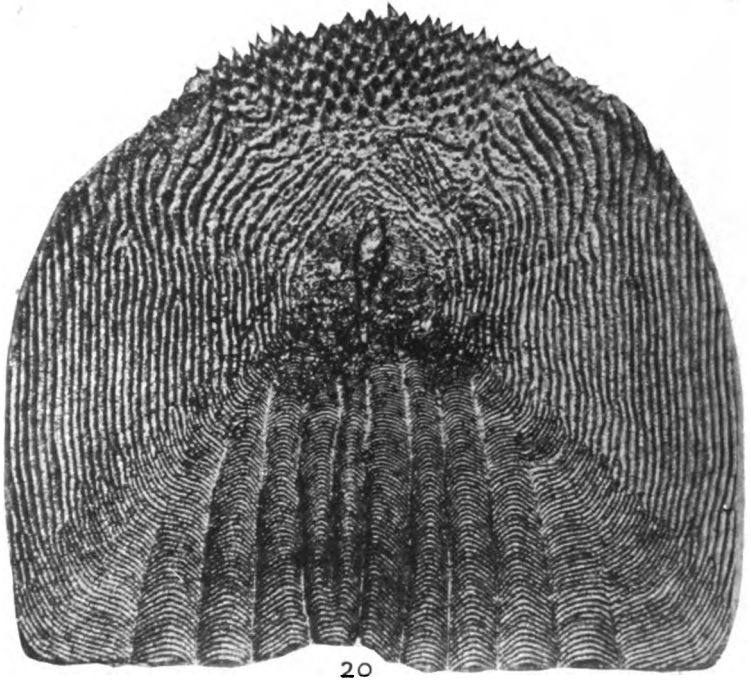

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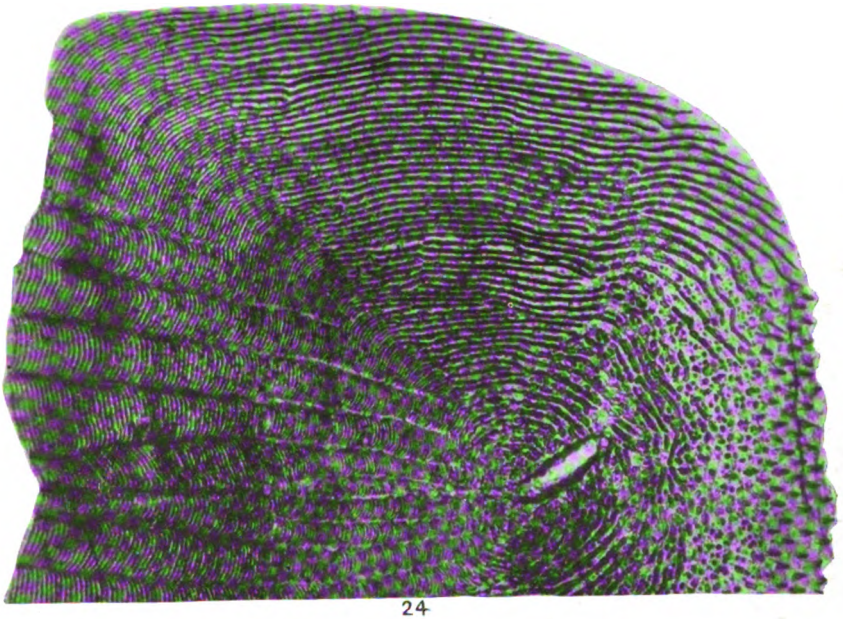

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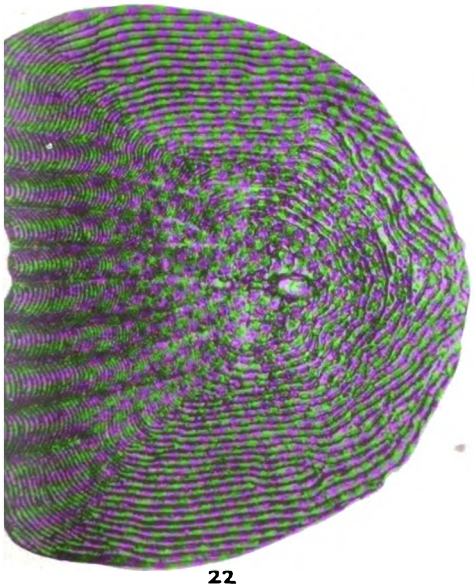

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PLATE XXIII

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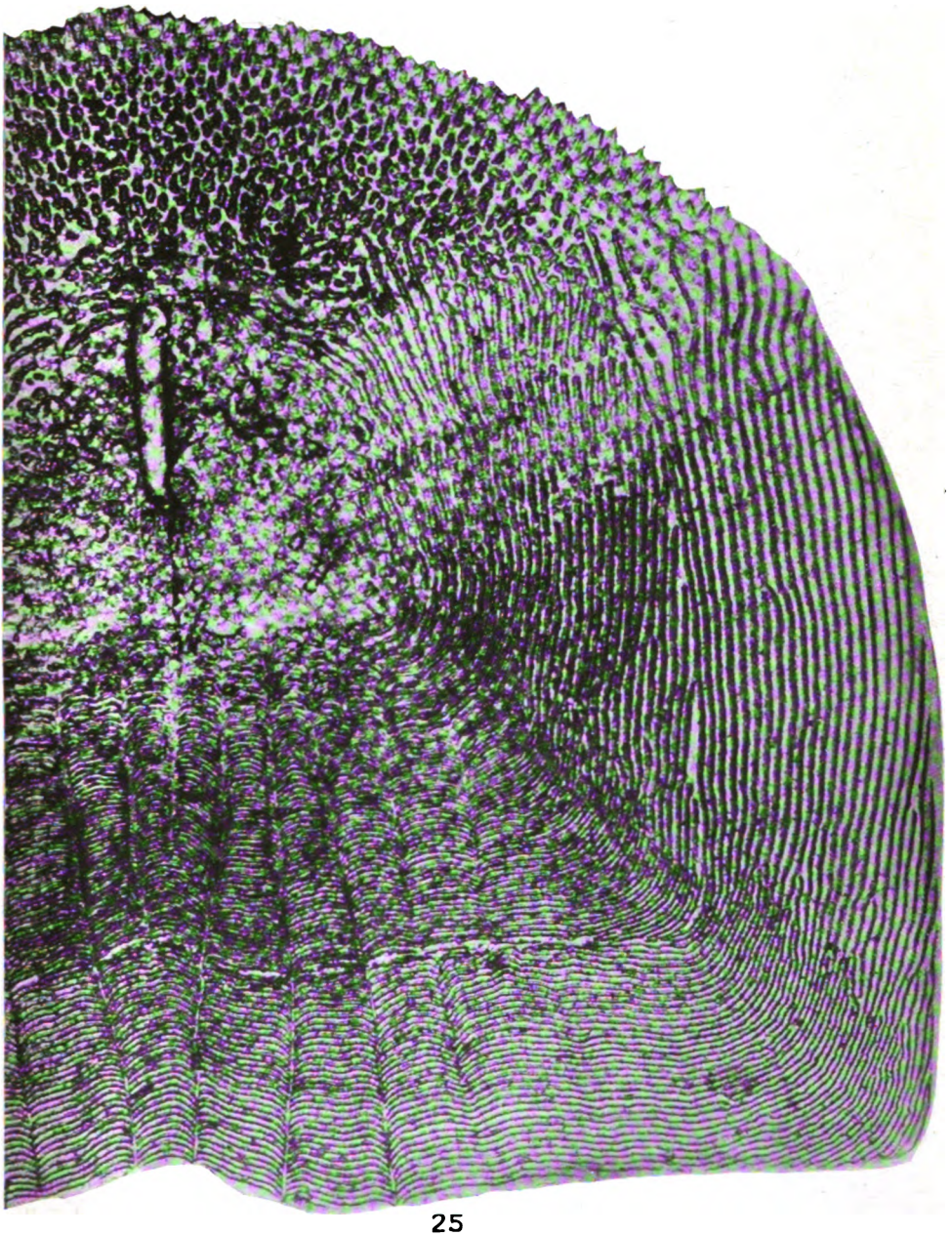

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PLATE XXIV JACOT

PLATE XXV

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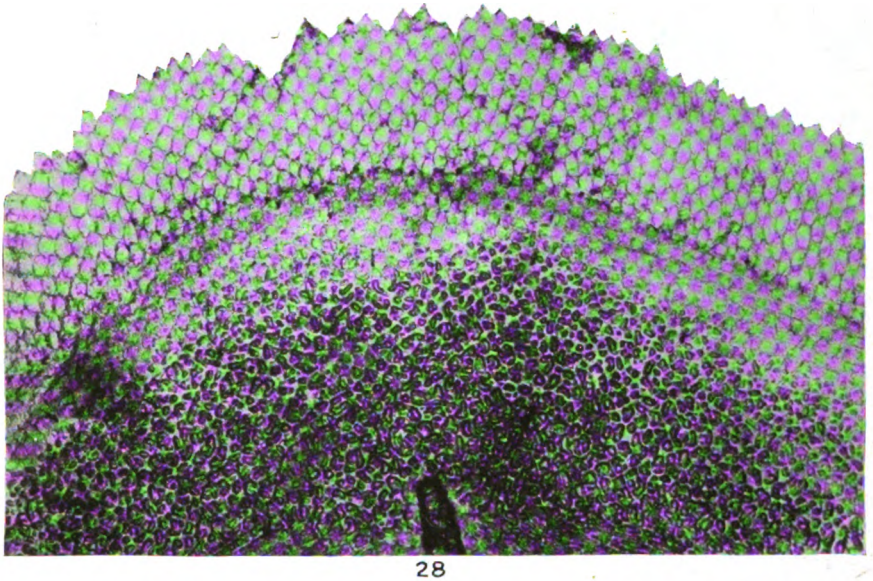

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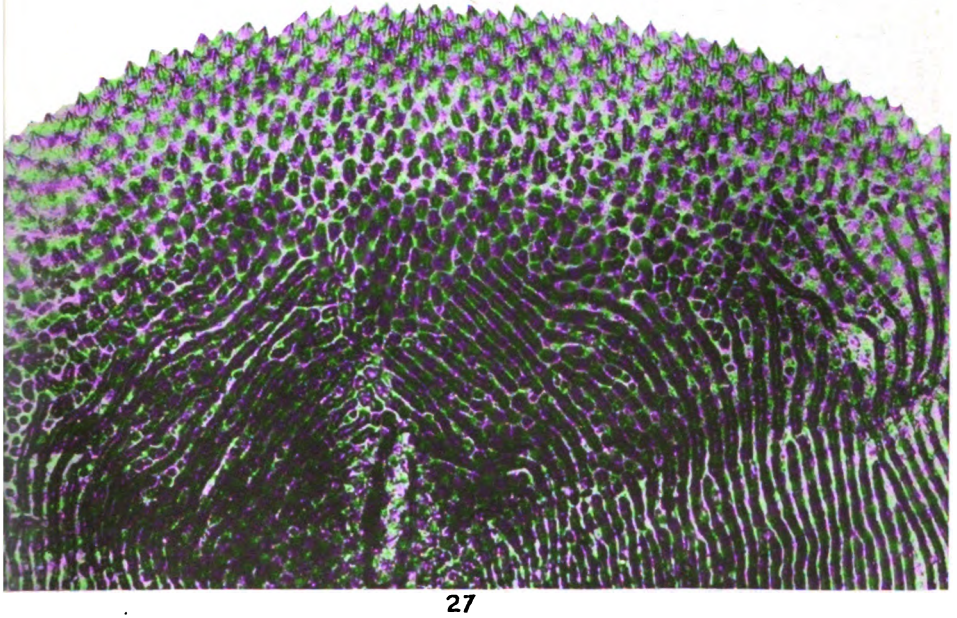

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PLATE XXVI

JACOT

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of the TRANSACTIONS OF THE AMERICAN MICROSCOPICAL SOCIETY, published quarterly at Menasha, Wis., required by the Act of August 24, 1912.

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George Banta, Publisher.

Sworn to and subscribed before me this 29th day of March, 1920.

[SEAL] Gertrude W. Sawyer,
Notary Public.

(My commission expires March 16, 1924.)

ORGANIZED 1878 INCORPORATED 1891

OCTOBER
1920

VOLUME XXXIX NO. 4

TRANSACTIONS

OF THE

AMERICAN MICROSCOPICAL SOCIETY

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Attention of members of the

AMERICAN MICROSCOPICAL SOCIETY

is called to the announcement of Grants from the Spencer-Tolles Research Fund.

(See TRANSACTIONS, July, 1913)

These awards are to stimulate and aid research in Microscopic work

1. **The Grantee must be a member of the Society.**
2. **Resulting discoveries must first be offered for publication in the Society Transactions.**
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The Secretary will consider it a favor if members will notify him of non-receipt of numbers of the TRANSACTIONS, and of changes of address.

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PAUL S. WELCH,
Secretary-Editor.

University of Michigan,
Ann Arbor, Michigan.

TRANSACTIONS

OF THE

American Microscopical Society

ORGANIZED 1878 INCORPORATED 1891

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EDITED BY THE SECRETARY

PAUL S. WELCH

ANN ARBOR, MICHIGAN

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TRANSACTIONS
OF
American Microscopical Society
(Published in Quarterly Instalments)

Vol. XXXIX OCTOBER, 1920 No. 4

MICRO-TECHNIQUE

Suggestions for Methods and Apparatus

N. A. Cobb
United States Department of Agriculture

I

SYSTEMATICALLY EXAMINING LARGE SERIES OF MICROSCOPICAL OBJECTS

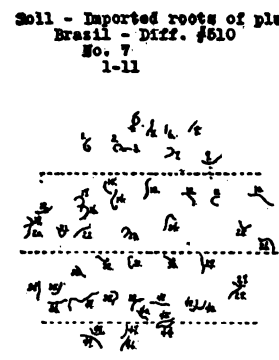
There are various methods of recording the position and character of each member of a large series of objects mounted on a microscope slide. One of the commonest methods involves the use of a recording, mechanical stage. Each object on the slide receives a record-number consisting of two separate readings from scales engraved on the mechanical stage. The following method, however, is successful without a mechanical stage or finder of any sort, and is characterized by simplicity and expedition. It may be called the method of charting.

The method consists in making a camera lucida drawing or chart, at low magnification, of all the objects of which it is desired to make record. The chart is diagrammatic; each object is represented on the chart by a simple, characteristic diagram, and the diagrams are then numbered in series. The sheet that carries the chart may also carry a series of printed numbers with corresponding spaces for records. (See Figure 1.) Where the objects belong to a few great groups, such as land-inhabiting, fresh-water, and marine, the printing of the blank sheets in correspondingly assorted colors is an advantage.

The chart is made by using a camera lucida and an objective of about five-inch focus.[1] In order to reduce the magnification, the objective may be screwed into the end of the draw-tube of the microscope barrel. A low power eye-piece is used with the objective, so

[1] A very strongly magnifying spectacle lens will serve the purpose.

that all the objects on the slide can be seen at one time. A chart having a magnification of five diameters is of convenient size. The suitable illumination is secured by using a concave mirror without sub-stage condenser. The light may be direct, in which case the objects are seen as dark bodies on a light background, or a dark-ground effect can be produced by inserting between the concave mirror and the objects a small opaque disc. A suitable disc may be made by stripping the barbules from a dark-colored six-inch wing or tail feather so



1	Tylenchus spiral.	26	See. 11
(2)	Cephalobus ?	27	" "
3	Doryl. styracturus	28	" "
4	Achromadora brasil	29	" "
5	Doryl. caudatus?	30	Mononchus minor
6	Elaphonema two	31	" fragment minor
(7)	Tylenchus perfectus	32	Rhabditis
8	Doryl. additicius	33	Y. Doryl. protrudens
9	" protrudens	34	See. 11
10	"	35	Y. Doryl.
11	?	36	Achromadora
(12)	Tropicanema tenuicolle	37	See. 11
(13)	See. 11 Egg	38	Rhabditis
(14)	Achromadora-papillae?	39	Elaphonema
15	Fibre	40	Y. Doryl.
16	Mononchus	41	See. 11
17	Achromadora	42	Fibre.
18	Rhabditis	43	See. 11
19	Ironus	44	Doryl. post
20	Elaphonema	45	Rhabditis
21	"	46	"
22	Rhabditis	47	Doryl. sl. 41.
(23)	Tylenchus	(48)	?
24	Mononchus minor	(49)	Doryl. sl. Eggs
25	Rhabditis	50	

Fig. 1. Record chart used in tabulating large numbers of microscopic objects arranged on a series of slides. As printed the chart was 5x8 inches, and carried only the two columns of figures 1 to 50 inclusive. At the left is seen the camera lucida drawing, or chart, recording the form, size, and relative position of forty-nine microscopic objects, —in this particular case, nemas. Immediately above the chart are seen the data relating to the particular slide charted, which was No. 7 in a series of eleven slides (1-11), and which carried a collection of forty-nine nemas gathered from soil attached to the roots of plants imported from Brazil. Names and other notes with regard to the nemas were typewritten opposite the appropriate numbers. Nos. 2, 7, 12, 13, 14, 23, 48, and 49 were encircled to indicate that these specimens were of especial interest. One-half size.

as to leave only a small fan-shaped tip at the end, from one-half to three-fourths of an inch across. With scissors, this is trimmed so as to have a somewhat rounded contour. While the right hand is engaged in making the chart, the left hand can flirt this little disc in and out between the objects and the concave mirror and so produce a

dark-ground effect as desired. To do this the feather "disc" must be materially smaller than the mirror.

The charts are nothing more than rude camera lucida drawings of the objects, and with practice can be made with great rapidity. A lot of fifty nemas mounted under a three-quarter inch round cover-glass can be drawn in two to three minutes with sufficient accuracy to make a very useful chart. (See Figure 1.) Each nema-diagram on the chart has four very distinct properties, (1) Position, (2) Form, (3) Size, (4) Orientation. For the most satisfactory work, it is desirable that a certain optimum number of objects exist on the slide. This optimum is determined by the number of them that will appear in a single field of the lens afterward used in searching. Suppose a sixteen millimeter objective is used as a searching objective, and a four millimeter for the examination; then the optimum number of objects under the cover-glass is that number which brings into each field of the sixteen millimeter objective one to three objects.

After the chart is made, the short, crooked lines, representing the nemas, say, are numbered in transversely arranged groups. Each transverse group of the series constitutes a band of nemas running across the mount and having such width as comes fairly well within the scope of a single field of the 16 mm. objective. These imaginary bands are illustrated in Figure 1. It will be seen that there are four such bands. The nemas are numbered more or less consecutively. Proceeding in this manner, on reaching the end of the first band, one numbers the second band, also more or less consecutively, and so on to the end.

In recording, begin with No. 1, placing it in the field of the 16 mm. objective. It is recognized by its size, form and orientation. Having recorded No. 1 and examined it with the 4 mm. objective, a glance at the chart will indicate at what distance, and in what direction, No. 2 lies from No. 1. Revolving to the 16 mm. objective and looking through the microscope at Nema No. 1, the slide is moved in the indicated direction until No. 2 is found and recognized. After recording No. 2, No. 3 is found in the same way, and so throughout. The novice will be surprised to find how easy it is, with a little practice, to follow the series through without error.

The drawings should be so made and numbered that the chart and the objects as seen under the microscope will resemble each other.

If no care be taken in this respect, the chart may be found to be "left-handed." Securing a "right-handed" chart is merely a matter of properly arranging the paper at the time the chart is drawn. Diagrams should be so made with reference to the printed matter that when it is right side up, the objects as viewed through the microscope will have the same orientations as the diagrams.

This completes the description of this method, except to explain that in the example illustrated, the numbers encircled are so marked in order to indicate that those particular specimens present noteworthy features.

The method may be elaborated in a variety of ways for the recording of nemas, rotifers, protozoa, desmids and a vast array of other microscopic objects. If the charts are of card-system size, say 5x8", they lend themselves to all sorts of convenient methods of filing. By using thin paper, carbon copies can be made at the original draft.

The charts can be made and used by a grade of assistant that might hardly be intrusted with the use of a recording mechanical stage, and who may lack training in the accurate reading of scales and the recording of numbers. Floating of the objects, of course, disarranges them. Newly made slides are sometimes subject to this disadvantage. The difficulty is avoided by keeping the slides always in a horizontal position.

II

OBJECT SUPPORT FOR A FREEZING MICROTOME

In this freezing microtome attachment, the object is to reduce the metal parts to a minimum and to concentrate the effects of the freezing mixture as much as possible upon the object to be frozen. To this end the object is placed on a thin metal plate, only about one to three thousandths of an inch thick, to which the necessary rigidity is imparted either by soldering it to a radiating framework in the form of a flat wheel sawed from somewhat thicker metal, or, preferably, by giving to the metal the form of a dome. These metals supports are illustrated in Fig. 2 and Fig. 3 in which they are shown full size. A six-spoked wheel, having a hub-hole one-eighth of an inch across,

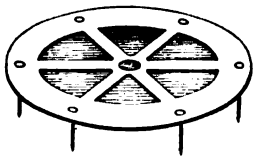


Fig. 2

is sawed from a sheet of German silver about one two-hundredth of an inch thick. The edges of the central aperture are beveled so that the mixture frozen on it becomes dove-tailed to the plate. In a similar way, the small, washer-shaped piece of German silver fastened to the top of the dome, as shown in Fig. 3, r, is also beveled.

The German silver wheel is soldered throughout to a round sheet of exceedingly thin brass or German silver. Then into six marginal perforations in the German silver wheel, brass pins are soldered, giving to the whole affair the appearance of a six-legged table. The heads of the pins are filed off so as to give clearance for the microtome knife. The pins serve to fasten the plate to a perforated cork, being thrust into the cork as shown in the illustration. The rim of the dome of thin sheet metal is somewhat similarly stiffened by soldering to it a ring of German silver which is perforated and supplied with six brass pins in the manner just described.

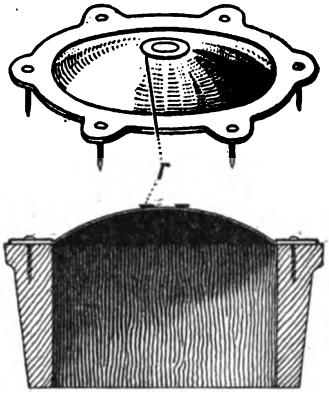


Fig. 3. Perspective view and longitudinal section of a freezing-microtome object-holder mounted on a cork cylinder. The holder is made of metal only about 2/1000 of an inch thick. The edges of the ring (r) are beveled so that the imbedding mixture when frozen is dovetailed to the holder.

Though the dome-form is somewhat more difficult to construct than the flat, it is more efficient for three reasons; It is more rigid, it gives a better clearance for the microtome knife, and it contains less material.

In the case of small and moderate sized objects of which only a few sections are required, the method is extraordinarily expeditious. Objects of such a size that they can be imbedded in a few drops of the freezing mixture placed on the control part of either of these metal supports can be frozen in a few seconds by applying an ordinary ether spray to the under side of one of these thin metal supports. The exceeding rapidity of the congelation gives rise to a consistency favorable to section cutting.

III

TO OBTAIN AN END VIEW OF A NEMA, ROTIFER, OR OTHER SIMILAR SMALL OBJECT

Suppose the object is a nema of which an end view of the head is required: decapitate the nema behind the pharynx with the aid of an eye knife, or similar very small tool, having a very slender, thin blade. The smallest and most slender-bladed knife used by oculists in operations on the eye is a very suitable tool, and it must have the degree of sharpness characteristic of surgical instruments in good order. Bring the nema by appropriate methods into glycerine; the decapitation should be done in a drop of glycerine placed on the surface of a transparent piece of celluloid. Push the nema to the bottom of the glycerine and against the celluloid; decapitate by pressing the edge of the knife against the nema as the latter rests on the celluloid. The celluloid is sufficiently soft so that the edge of the knife will not be dulled. If the knife is sharp, the cut will be clean, and the object satisfactory. If the knife is dull, the nema will be more or less crushed at the point of section and the preparation may prove unsatisfactory.

Mount the head in melted glycerine jelly, using sufficient jelly so that the object may stand on end after being covered in. Place the mount on the stage of a microscope, bring the object into focus, and with a dissecting needle gently shove the cover-glass slightly back and forth until the object is seen to be on end. Allow to remain on the stage of the microscope until the jelly sets, watching from time to time to see that the object maintains the desired position.

According to my experience, this is a better method of obtaining end-on and sectional views of the heads of free-living nemas and other similar small organisms than that of sectioning and imbedding. The trouble with the method of sections is that the microtome knife very seldom cuts the object to advantage. It is quite likely to cut in the wrong place. If the ends of the setae or the surfaces of the lips are removed in the first cut, it is a very troublesome matter to obtain a good view or good sketch of the structures. Even if some of the parts should not be lost or offer difficulty in mounting, there are so many chances that the microtome blade will cut through at a disadvantageous place that, as a rule, a very considerable number of nemas will have to be sectioned before a good preparation is secured.

The method of sections has the further disadvantage that the following of such small objects through the various dehydrating and

staining fluids, and the final orientation of them, is a tedious and difficult matter. Moreover in the case of nemas, there is considerable difficulty in properly imbedding the object. The cuticle of nemas is so impenetrable that unless special precautions are taken, the paraffine will not thoroughly penetrate the tissues, and the results will be unsatisfactory.

End views may be obtained by mounting the nemas in a microscopic well made from a thin section of thermometer tubing. The tubing should be like that used in the most delicate medical thermometers, that is to say, with the smallest aperture procurable. This tubing may be bought under the name thermometer, or barometer tubing. It is well to have on hand ground sections of varying thickness, from one-quarter of a millimeter thick to one millimeter or more. The discs are cemented to a glass microscope slide at the time of using by means of smoking hot wax or other suitable cement. Before cementing the disc to the slide, fill the capillary aperture in the disc with mounting fluid. This may be easily done by placing on the slide a very tiny drop of the mounting fluid, and laying the disc onto the small drop. The mounting fluid will enter the aperture by capillarity. If it be desired to look at the head end of a nema, it is placed in the microscopic well, tail down. If the nema is too long for the well, it may be cut to fit it. The point is, to see that the object has about the same length as the depth of the well, so that the end portion of the object it is desired to view will come close to the under side of the cover-glass when this latter is placed on the top of the well, or rather on the disc of the glass containing the well. In placing the nema in the well, a suitable tool is a small, curved hair cemented to the end of a dissecting needle. Human eye-brow hairs are suitable for this purpose. Using this method, the specimen can be examined in clove oil, cedar oil, or any mixture of these or any other similar thin mounting fluid. Cedar oil, having the same refractive index as the glass composing the well, has advantages in connection with illumination. The illumination in aqueous media is less satisfactory.

When the glass discs are not in use, it is best to keep them in absolute alcohol in a glass-stoppered bottle. They should not be allowed to become dry with mounting fluid in the capillary orifice, otherwise they will be very troublesome to clean out.

IV

DESTAINING OF NEMAS OR OTHER SMALL OBJECTS IN THE DIFFERENTIATOR

In handling a mass of small organisms by the differentiator method, there is sometimes considerable difficulty in securing satisfactory destaining. There is little difficulty in getting a mass of organisms thoroughly impregnated with the stain, no matter how varied they may be in species and in size; it is simply a matter of time. The trouble comes in destaining. If the destaining process is carried on until the largest of the objects, or the most impenetrable ones, are sufficiently destained, it will generally happen that smaller specimens, or those more easily penetrated, are deprived of too much of their color. It is therefore a matter requiring considerable experience and judgment to successfully destain such a miscellaneous collection. The difficulty is considerably increased by the fact that when enclosed in the differentiator tube, the specimens are not very easy to examine critically by any ordinary method. If the differentiator be held toward a strong light, the organisms may be examined by the aid of an ordinary pocket lens, but not very critically. The most satisfactory piece of apparatus for this work is what is sometimes known as the chemical microscope, in which the objective is below the stage and the light that passes through it from above is reflected by a prism placed below so as to pass obliquely upward through a barrel carrying an eye-piece. If the differentiator tube containing the destained nemas is laid on a glass stage over the objective of such an inverted microscope, and a little water, or still better, cedar oil, be placed between the differentiator tube and the glass stage, it will be found that the nemas or other objects will sink to the bottom of the fluid in the differentiator tube so as to come as near as possible to the objective of the microscope. If the glass stage is thin, there is no difficulty in using a one-half to two-thirds inch objective. In this way, the nemas may be examined more critically with regard to the extent of the destaining.

If it is desired to use a lens of higher power, it is sometimes possible to do so by resorting to another method. Place a cover-glass on a horizontal surface, and on the cover-glass a good-sized drop of cedar oil. Lay the differentiator tube into this drop of cedar oil in such a way that the nemas come opposite the cover-glass. It will now be

found that the cover-glass will adhere to the differentiator by capillarity, so long as the differentiator is held in a horizontal position. If the chemical microscope stage has a large aperture, it will be possible to lay the differentiator across the stage, cover-glass downward. In this way, if the differentiator tubing is thin, it will be possible to use even quarter-inch objectives of long focus.

Where considerable work is done with differentiators, a chemical microscope used in this way is a valuable accessory.

V

COMPRESSORIUM FOR CHROMOSOMES

When chromosomes or other similar minute bodies are so massed together that one lies behind another and is thus liable to be missed in counting, the compressorium described below may prove useful in overcoming the difficulty, which none of the ordinary compressoria will do.

When such a mass of chromosomes is flattened out by pressure, the individual chromosomes behave somewhat as would the seeds of a pulpy fruit under similar circumstances. They appear to be of a different consistency from the material in which they lie, and behave under pressure as if harder and more compact than the surrounding matter. Under moderate pressure they do not show much tendency to break in pieces, but rather to accommodate themselves to the narrower quarters by rearranging themselves more nearly in one plane. So far as enumeration of the chromosomes is concerned, this new arrangement has two advantages: 1st, they may all be more readily brought into a single view, that is, all brought into focus at one time; 2nd, in the flattening-out process, they slip one over another somewhat, and recede from each other—for instance, as the seeds inside a grape will do, when similarly pressed.

Fig. 4. Two curved, perforated, steel springs made from thin, safety-razor blades, as described in the text. These two forms, while of the same length, nearly one inch, are of different degrees of springiness; that at the left being the weaker.

The compressorium I have devised to secure this effect is constructed as follows: Take a safety-razor blade—one of the thinnest kind, having perforations an eighth of an inch in diameter—and

soften it by heating it to a red heat. With shears, cut a somewhat diamond-shaped piece from the softened blade, so that the "diamond" is about three to four times as long as wide, and has one of the round apertures in its center; bend this elongated "diamond" into a symmetrical bow whose depth is one-eighth of an inch or more. See Fig. 4. Heat the bow in a flame to a cherry-red and plunge it into cold oil or water to harden it. This will result in a springy piece of metal that can be utilized to exert pressure on a small cover-glass under which are mounted cells containing the chromosomes it is desired to scatter. The length of the piece of springy steel may conveniently be made to be about one inch, so that it will just reach across an ordinary three-by-one glass microscope slide. Bind the slide in a piece of thin metal having a three-quarter inch perforation at the back—that is to say, so bend a piece of thin sheet metal that an ordinary slide will slip into it through grooves along the two sides of the folded piece of metal. See Fig. 5. This metal should simply pass around the edges of the slide and lap over about a sixteenth of an inch at each edge leaving one face of the slide uncovered. The grooves should be a little wider than the thickness of the slide—at least enough wider so as easily to admit the thin perforated metal spring. Place the cells, the chromosomes of which are to be studied, on the slide opposite the middle of the three-quarter inch aperture. Use very little mounting medium; cover the cellular tissue to be treated with a small round cover-glass. Tuck the ends of the bowed piece of springy perforated steel under the edges of the metal slide-case or holder, holding the spring against the small cover-slip in such a way that the cells to be compressed lie opposite the center of the small perforation. Press and lock the spring in the same way as in the case of the springs at the back of an ordinary photographic printing frame. The cells will now be under pressure at or near the center of the perforation in the steel spring. The entire contrivance will differ but very

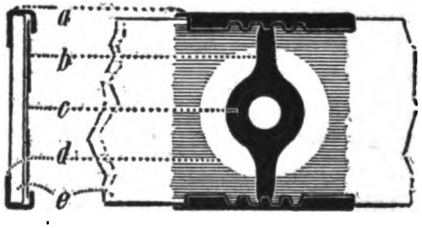


Fig. 5. Portion of a 3x1 inch glass microscope slide enwrapped with thin metal as described in the text. *a*, thin metal wrapper; *b*, one of the springs shown in Fig. 4, placed in position on the slide so as to press the small round cover-glass, *c*, against the slide, *e*; *d*, aperture in the back of the metal wrapper, *a*. The ends of the spring, *b*, enter through the notches on the edges of the wrapper, *a*, so that in being applied the spring does not need to be rotated more than a few degrees.

little in form and size from an ordinary microscope slide and can be placed on any microscope stage in the same way as a slide. The piece of springy steel is so thin that it in no way prevents the use of a high-power immersion objective. Needless to say, it is for this reason that it is made from such thin metal. The spring may be manipulated with the aid of matches or wooden toothpicks.

Ordinary slides and cover-glasses are almost never perfectly flat. Better results will be obtained by this method if the slide has its convex surface up and the cover has its convex surface down, so that the cellular tissues to be treated lie between two very slightly convex surfaces. It will be found that in this way very compact groups of chromosomes and other similar objects can sometimes be scattered so as to be counted, when otherwise they could not be counted.

There seems to be comparatively little danger of exerting too much pressure. The beginner's tendency at first is to exert, if anything, too little pressure. The greatest difficulty arises from sliding the glasses on each other, since much of this ruins the preparation. To overcome this difficulty, a series of three or four notches, close together, may be filed in the edges of the metal holder before it is folded about the slide,—or rather about the metal core on which it is bent, or formed, and which naturally has a little greater width and thickness than the slide. If now the bowed spring has a length a little less than the distance between the bottoms of the notches in the edges of the slide-holder, it will be found when it is pressed down that the pointed ends can be tucked through the notches and under the edges of the holder without materially sliding or rotating the spring. The accompanying illustrations will assist in understanding this simple and effective device.

The particular cells to be compressed are prepared and searched out in the usual way, then dissected out together with as little of the surrounding tissue as possible, an operation performed with the aid of an ordinary dissecting microscope. It may be advisable to look at the group of chromosomes from both sides. To do this, the metal holder, instead of having a three-quarter inch perforation, should have a much smaller perforation, say about one-eighth of an inch. Instead of using a three-by-one glass slide, cement to the inside of the metal holder a thin cover-glass several sizes larger than that to be

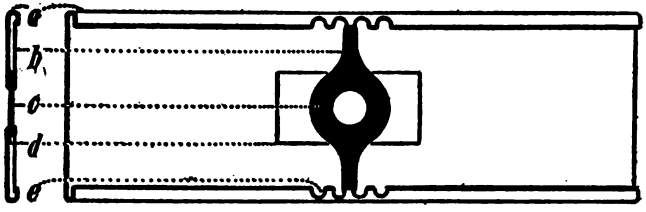


Fig. 6. A metal holder for clamping a microscopic object between two thin cover-glasses. *a*, metal holder; *b*, steel spring as illustrated in Figs. 4 and 5; *c*, small, round cover-glass; *d*, rectangular cover-glass underneath the round cover-glass; *e*, notches in the metal holder for the reception of the spring. This holder enables the microscopist to look at the object with an immersion lens from either direction.

placed over the object. As the metal holder, in order to be stiff enough, has to be several times thicker than the bowed spring, it may be advisable to bevel the edge of the round aperture in the holder, so that it will interfere as little as possible with the use of an immersion objective. On a slide constructed in this manner, the object is held between two cover-glasses, and hence may be viewed from either side with equal ease. Such a slide furthermore permits the use of an immersion lens as a condenser, a proceeding that has advantages.

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